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STRUCTURE AND GENETICS OF GLUTEN PROTEINS IN RYE, WHEAT AND RELATED SPECIES

by

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ABSTRACT

Gliadins from wheat and rye, prepared by Osborne fractionation, were separated by gel filtration chromatography in a strongly dissociating solvent into four fractions of average molecular weights of 100,000, 44,000, 27,000 and 10,000. The wheat and rye gliadins showed large variations in the distribution of protein among the four fractions. Over 50% of the rye gliadins had molecular weights of approximately 100,000 while in wheat the majority of the gliadins had molecular weights near 40,000.

Gliadin fractions obtained by gel filtration were examined by amino acid analyses and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Corresponding fractions from different wheat varieties had similar amino acid compositions. The gliadin fractions of molecular weights 100,000, 40,000 and 27,000 had amino acid compositions similar to whole gliadin while the fourth fraction (MW 10,000) had an amino acid composition similar to wheat albumin. In contrast the rye gliadin fractions showed a trend of increasing glutamic acid and proline content as molecular weight increased. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that three hexaploid wheat varieties studied had identical subunit structures while a tetraploid wheat variety lacked three high molecular weight subunits present in the hexaploids. The rye gliadins contained only three subunits.

Gliadins and glutenins extracted from whole seeds of Triticum aestivum (AABBDD), Aegilops squarrosa (donor of D genome), Triticum monococcum (donor of A genome), and Secale cereale were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Seven gliadin and nine glutenin subunits in Triticum aestivum were also present in both Triticum monococcum (AA) and Aegilops squarrosa while five gliadin and five glutenin bands appeared to be inherited from only one of the two diploids studied. S. cereale had fewer gliadin and glutenin subunits than Triticum monococcum or Aegilops squarrosa but three of the four gliadin and eight of nine glutenin subunits present in S. cereale had molecular weights identical to subunits present in Triticum monococcum and/or Aegilops squarrosa. This similarity in subunit structure suggested that these three diploids were phylogenetically related.

Mixing studies of a synthetic dough system consisting of gliadin, glutenin and starch showed that the high molecular weight gliadin fraction isolated from a hard red spring wheat (cv. Manitou) by gel filtration strengthened the mixing curve while the low molecular weight gliadin fractions did not. These results indicated that the molecular weight distribution of gliadins in natural flour systems might be important in determining rheological properties.

An analysis of the insoluble residue proteins of a hard red spring wheat (cv. Manitou), following reduction and alkylation, showed that the majority of these proteins were soluble in 70% ethanol and 0.1N acetic acid. Analysis of these fractions by amino acid analysis, electrophoresis

and sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that these proteins were similar to glutenin.

In addition a hypothesis has been presented to account for the natural selection of high levels of glutamine and proline in cereal endosperm proteins.

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INTRODUCTION

The factors responsible for the unique viscoelastic properties of wheat and rye doughs which allow the production of leavened bread reside in the properties of their gluten proteins. Fractionation and reconstitution studies indicate that the gluten proteins soluble in 70% ethanol (gliadin) are mainly responsible for differences in the loaf volume potential of bread wheat flours while the glutenin proteins are mainly responsible for mixing properties.

Although the physical and chemical properties of the gliadins have been widely studied, the factors responsible for loaf volume potential have not been determined. However one area where these studies are lacking is in the qualitative and quantitative distribution of gliadin components based on molecular size. Thus the major objective of this thesis was to study inter- and intravarietal differences in wheat and rye gliadins on this basis.

In the present study the initial basis of separation was gel filtration. This method is useful for the separation of proteins on the basis of molecular size in fairly large quantity. The physical and chemical properties of the gliadin fractions separated on this basis could then be further characterized. One technique that was useful in characterizing fractionated gliadins was polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS), which

allowed the determination of molecular weights of the various components. Gliadin fractions were also characterized by amino acid analysis and electrophoresis.

Although the effects of individual gliadin fractions on baking properties could not be determined due to lack of large enough samples, a study of the effects of different sized gliadin fractions on the mixing properties of a synthetic dough system were studied. This study was possible due to the recent introduction of the electronic recording dough mixer in which small samples could be tested.

In order to improve the quality of bread wheats it may be useful to have a better understanding of the inheritance of and factors directing the evolution of their proteins. In the present study an attempt has been made to determine the inheritance of gliadin and glutenin subunits in wheat. In addition a hypothesis has been put forward to account for the amino acid composition of cereal proteins on the basis of natural selection.

The format which has been adopted in this thesis is somewhat different than is customary. The section usually dealing with the results and discussion has been replaced by six papers written in a style suitable for publication in Cereal Chemistry. This method has several advantages. It allows the early publication of results without extensive rewriting and allows the author to write in the style which will be most useful to him in the future.

LITERATURE REVIEW

The protein content of wheat varies from approximately 7 to 18% depending on variety, environment and soil conditions. Of the total protein of the seed, the endosperm contains approximately 72%, the bran 20% and the germ 8%. The bran and germ have higher relative concentrations of protein by weight. Flour, the main product of wheat, consists almost entirely of endosperm tissue with protein contents varying from 7 to 17%. Other major components of flour include carbohydrates (approx. 70%), lipids (approx. 2%) and water (approx. 17%).

Upon addition of water to wheat flour followed by mixing, the gluten proteins become hydrated and form a viscoelastic mass which provides a framework in which the other components are held. This viscoelastic mass has the property of retaining gas and thus makes possible the manufacture of leavened bread.

The quality of bread produced from wheat flour depends both on the quantity and properties of the proteins present. Finney and Barmore (1) have shown a direct correlation between loaf volume and protein content in a large number of hard red winter and spring wheats. The increase in loaf volume as protein content increased was found to be linear between 8 and 18% for a wide variety of wheats varying in baking quality. Loaf volume changes of up to 15% per 1% change in pro-

tein content were found in some varieties.

The composition of the proteins present in wheat flour has also been shown to be important in the quality of bread produced. Although several methods of fractionating and classifying wheat flour proteins have been investigated, the classic method of Osborne (2) is still the most widely accepted. In this method the flour proteins are sequentially extracted with saline water, 70% ethanol and dilute acid. Five protein fractions are obtained as defined by their solubility. A definition of gluten is also included:

Albumin - soluble in water and dilute salt solutions

Globulin - soluble in dilute salt solutions but insoluble in water

Gliadin - soluble in 70% ethanol solution

Glutenin - soluble in dilute acid solutions

Residue - insoluble in all of the above solutions

Gluten - the proteins remaining after extraction of flour with water or dilute salt solutions.

Physical and Chemical Properties of Endosperm Proteins

1. Albumins

Pence et al (3) isolated the salt soluble proteins from 32 flours differing widely in baking quality and found that these proteins accounted for 13 to 22% of the total protein. Of the salt soluble protein approximately 60% were albumins and 40% were globulins. The molecular weight of the albumin fraction was reported to be 28,000 but in disaggregating

solvents this value fell to 17,000 (4). Feillet and Nimmo (5) isolated two albumins, 13A and 13B. 13A had a high alanine content while 13B had a high valine content. Sedimentation equilibrium gave molecular weights of 24,800 for 13A and 13,950 for 13B. Up to 21 electrophoretically distinct albumins have been reported (6).

The effect of the albumin fraction on baking quality has been studied by a number of workers. Hoseney et al (7), using reconstitution techniques, concluded that albumin concentration had little effect on loaf volume but did find that components present in the albumin fraction contributed to gassing power and handling properties of the dough. However it was concluded that these effects were probably due to glycolipids present in the albumin fraction.

Several workers have shown that the water soluble fraction in flour may cause small variations in water absorption (7,8). Recently Orth and Bushuk (9) showed that the ratio of albumin to globulin was positively correlated with loaf volume per unit protein.

2. Globulins

Globulins make up 5 to 11% of the total protein in wheat flour (3). Pence and Elder (4) isolated three globulins from wheat flour that were characterized by low tyrosine and amide nitrogen and high arginine. Recently Silano et al (10) obtained 15 electrophoretically distinct globulins. Starch-gel electrophoretic studies of eight wheat varieties varying in baking quality showed little variation in globulin patterns (11). Although the role of globulins in baking quality has

not been studied extensively, studies by Pence (12) and by Orth and Bushuk (9) shows a negative correlation between loaf volume and the ratio of globulin to albumin per unit protein.

3. Gluten

Gluten was first isolated in 1728 by Beccari (13). In general gluten is isolated by gentle washing of dough in excess water or dilute salt solutions. Recently Mauritzen and Stewart (14) have described a method of isolating gluten by the centrifugation of a dough suspension. Seven layers were obtained, the third being gluten.

Dry gluten contains 75 to 85% protein, 5 to 10% lipid and occluded starch, and is capable of binding twice its weight of water (15). Gluten proteins consist mainly of gliadin, glutenin and residue proteins which make up approximately 40 to 45%, 15 to 20% and 30 to 40% of the total protein respectively. Woychik et al (16) also found that gluten contained approximately 7% salt soluble protein.

Several solvents for extracting gluten proteins have been described, the most common being aqueous acetic and lactic acid. These solvents dissolve approximately 80% of the gluten proteins (17). Hydrogen-bond disrupting solvents such as aqueous urea (18) and aqueous sodium salicylate (19) also extract high proportions of gluten protein. Recently up to 95% of the total flour protein has been dissolved in the AUC solvent of Meredith and Wren (20). This solvent consists of acetic acid (0.1M), urea (3M) and cetyltrimethyl ammonium bromide (0.01M).

The amino acid composition of gluten and its component proteins (22,23) is shown in Table 1. These proteins are characterized by high contents of proline and glutamic acid and a relatively high proportion of non-polar amino acids. A high proportion of the glutamic acid content in wheat proteins has been shown to occur as glutamine (24). In addition these proteins possess a low number of ionic amino acids. All these factors probably contribute to the strong aggregation tendencies of gluten proteins (15).

The high proportion of glutamine residues gives gluten proteins a high hydrogen-bonding potential. A number of studies have demonstrated the importance of hydrogen-bonding in relation to dough properties. Barney et al (25) found that acetylation of amide groups in gluten proteins destroyed the cohesiveness of dough. In another study gluten strength was improved by substituting deuterium oxide in place of water (26). The increased dough strength could be attributed to the stronger hydrogen bonding energy associated with deuterium.

The high proportion of non-polar residues (approx. 50%) in gluten proteins is generally thought to be important in stabilizing gluten structure by hydrophobic bonding. Dough structure is destroyed by a variety of solvents which disrupt hydrophobic bonds (27,28). From the data of Woychik et al (29) the hydrophobicities of gliadin and glutenin were calculated to be 1,109 and 1,016 cal. respectively, values high enough to stabilize gluten complexes by this type of bonding (15).

Table 1. Amino Acid Composition of Gluten, Gliadin and Glutenin[a] (moles amino acid per 10^5 g)

Amino Acid	Gluten	Gliadin	Glutenin
Arginine	20	15	20
Histidine	15	15	13
Lysine	9	5	13
Threonine	21	18	26
Serine	40	38	50
Aspartic Acid	22	20	23
Glutamic Acid	290	317	278
Glycine	47	25	78
Alanine	30	25	34
Valine	45	43	41
Leucine	59	62	57
Isoleucine	33	37	28
Proline	137	148	114
Tyrosine	20	16	25
Phenylalanine	32	38	27
Tryptophan	6	5	8
Cystine/2	14	10	10
Methionine	12	12	12
Ammonia	298	301	240

[a]Data of Wu and Dimler (22,23)

Although the high proportion of proline in gluten proteins is not directly involved in the aggregation of these proteins, the effect on secondary structure by this amino acid probably contributes to this property. The fact that gluten proteins have less secondary structure would increase inter-chain interaction at the expense of intra-chain interactions. Several workers using optical rotatory dispersion and circular dichroism have shown that these proteins have very low percentages of α-helical structure. Wu and Cluskey (30) calculated α-helical contents of 24 and 13% for gliadin and glutenin respectively in 0.002M hydrochloric acid.

Although the content of cysteine and cystine in gluten is only of the order of 1.4 molar percent, these amino acids are thought to be of extreme importance in gluten quality. Disulfide bonds between polypeptide chains increase mixing resistance while disulfide exchange reactions involving sulfhydryl groups give dough mobility (31-33). Belderok (34) calculated on optimum ratio of fifteen for disulfide versus sulfhydryl bonds in dough for best baking performance. Kuchumara and Strelnikova (35) found that the disulfide-sulfhydryl ratio was greater than seven in strong mixing flours and that this ratio increased during flour storage. Tsen and Bushuk (36) found that the mixing strength was inversely related to the number of reactive sulfhydryl groups. However during mixing apparently only a small percentage of disulfide bonds are broken (37).

4. Gliadin

Gliadin, the alcohol soluble component of wheat flour protein, makes up approximately 40 to 45% of the total flour protein (2,9,13). Gliadin is usually isolated from flour or gluten with a 70% aqueous ethanol solution by the classic Osborne procedure (2). Another common method is that of Jones et al (38). An 0.1M acetic acid extract of gluten is neutralized and ethanol is added to produce a 70% alcohol solution. After centrifugation, the gliadin remains in the supernatent while the precipitate consists of glutenin. Other methods of gliadin isolation include extraction with 35% i-propanol (39) and gel filtration chromotography (40). However gliadins isolated by these different procedures may not be strictly equivalent.

Molecular weights of wheat gliadins obtained by gel filtration and sedimentation equilibrium vary between 25,000 and 50,000 (41-43). However Beckwith et al (40) have isolated a gliadin fraction by gel filtration which has a molecular weight greater than 100,000. This fraction accounts for approximately 7% of the total gliadin in hard red winter wheats. On reduction this fraction had a molecular weight near 40,000. Recently by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, Bietz and Wall (44) have shown that hard red winter wheat gliadins contain major gliadin subunits of molecular weights 36,500 and 44,200.

Extensive attempts have been made to correlate electrophoretic patterns with gliadin quality. Elton and Ewart (11) using starch gel

electrophoresis found wide variations in gliadin patterns. Doekes (45) studied 84 varieties of wheat varying widely in baking quality. He was able to categorize their gliadins into five general electrophoretic patterns. Poor quality soft winter wheats had compact patterns while spring wheats of good baking quality had scattered patterns. However wheats in the same class varying in breadmaking quality had similar gliadin patterns (45,46). Recent studies now indicate that within the bread wheats the electrophoretic patterns of the gliadins do not correlate with baking quality (9,46).

Several studies have shown that there is little variation in the amino acid composition of gliadins from different wheat varieties (47, 48). Gliadin is characterized by high levels of glutamic acid (of which approximately 85% is glutamine (47)), of proline and of non-polar amino acids. The high glutamine content probably accounts for the aggregation tendencies of gliadin while the high content of non-polar amino acids is probably responsible for its solubility in organic solvents. The lack of ordered structure in gliadin is probably due to the high proline content (47).

A number of studies have shown that the gliadins are important to breadmaking quality. Finney (49) found a significant positive correlation between loaf volume and the ratio of gliadin to glutenin. A similar result was obtained by Orth and Bushuk (9) in a study of 28 wheat varieties varying in baking quality. Shogren et al (17) solubilized gluten in 0.005 M lactic acid and studied the influence of various fractions isolated

by pH precipitation on baking quality. When added to dough, the fraction soluble at pH 5.6 (gliadins) increased extensibility, loaf volume and oxidation requirement and decreased mixing time and water absorption. Hoseney et al (50) using reconstitution techniques showed that the gliadin fraction was responsible for differences in loaf volume potential between good and poor-quality varieties.

5. Glutenin

Glutenin is defined as the acid or alkali soluble component of wheat flour proteins remaining after salt and alcohol extraction. It accounts for approximately 15 to 25% of the total flour proteins (2, 9,13). Several methods are available for isolating glutenin. In the classic Osborne procedure (2) glutenin is isolated from flour by solubilization in 0.1M acetic acid after prior extraction with dilute salt solution and 70% ethanol. Another common method is the procedure of Jones et al (38). A 0.1M acetic acid extract of gluten is neutralized and made 70% in ethanol. The precipitate contains the glutenin. Meredith and Wren (20) described a method of glutenin isolation by gel filtration of flour proteins solubilized in the dissociating solvent, AUC.

The glutenins vary in molecular weight from 100,000 to over 2,000,000 (38,51,52). However reduction of disulfide bonds drastically reduces the molecular weights of these proteins to less than 100,000 (51,53,54). Unreduced glutenin moves as a single band in moving boundary electrophoresis (38) and only a small proportion migrates in starch or

polyacrylamide gel (55). In contrast, reduced glutenin has electrophoretic patterns similar to gliadin (53). This and other evidence (56-58) suggested that glutenin might be a polymer of gliadin. However more recent evidence indicates that this is not true. Bietz and Rothfus (59) found that although enzymatic hydrolysis of glutenin and gliadin gave some common peptides, most were unique. Bietz and Wall (44), by SDS-polyacrylamide gel electrophoresis, have shown that most glutenin subunits do not correspond to those in gliadin. At least fifteen distinct glutenin subunits were identified ranging in molecular weight from 11,600 to 133,000.

Beckwith and co-workers (60,61) studied the effects of reduction and reoxidation of gliadin and glutenin. Reduced gliadin reoxidized more rapidly than reduced glutenin. At low protein concentrations (1%) reduced gliadin, upon oxidation, regained its original properties with little inter-chain disulfide bonding while glutenin gave high molecular weight proteins indicating reformation of inter-chain disulfide bonds. At 5% concentrations the reoxidation of gliadin yielded glutenin like proteins which were cohesive but lacked elasticity, while reoxidized glutenin proteins were both cohesive and elastic. Reduction of native glutenin led to an initial rapid decrease in viscosity followed by a slight increase. The initial decrease was attributed to the breakage of inter-chain disulfide bonds while the later increase in viscosity was attributed to the breakage of more resistant intra-chain disulfide bonds, allowing unfolding of the polypeptide chains.

Reconstitution studies (17,50) have shown that glutenin controls the mixing requirement in doughs. Several studies have tried to correlate baking quality with glutenin content. Orth and Bushuk (9) found that the glutenin content was positively correlated with mixing tolerance index and negatively correlated with dough development time and loaf volume per unit protein. High contents of glutenin were associated with weak flours of poor baking quality. Tsen (62) found that hard wheats of good baking quality had a lower content of glutenin than soft wheats of poor quality.

6. Residue Proteins

Exhaustive extraction of wheat flours with dilute acetic acid yields a highly hydrated residue which contains a substantial amount of the total flour proteins (63,64). Orth and Bushuk, (9) comparing the protein solubility distribution of 26 wheat varieties of diverse baking quality, found that the residue proteins accounted for 20 to 40% of the total flour proteins.

Very little data is available on the chemical and physical properties of these proteins. Cluskey and Dimler (65) solubilized most of the acetic acid insoluble flour proteins in 70% chloroethanol -0.1N hydrochloric acid. Reduction and alkylation of these proteins gave polypeptide chains with electrophoretic mobilities similar to wheat albumins. Inamine et al (66) solubilized about 85% of the flour residue proteins in 0.1M aqueous dimethylaminoethanol. Two high molecular weight fractions were obtained by gel filtration on agarose. Reduction

and alkylation of these fractions gave polypeptide chains with electrophoretic mobilities similar to reduced and alkylated glutenin. Mecham and Co-worker (67,68) solubilized most of the residue protein with 0.04 mM mercuric chloride in 0.01M acetic acid. In seven flours, residue protein accounting for 12 to 28% of the total flour protein was solubilized. Recently, by SDS-polyacrylamide gel electrophoresis, Orth and Bushuk (unpublished data) found that flour residue proteins contained subunits with molecular weights similar to glutenins.

The relationship between the residue proteins and baking quality has been studied by a number of workers. Pomeranz (69) found that poor baking quality wheat flours had more protein dispersible in 3M urea and less insoluble protein than good quality flours. Dronzek et al (48) found that the lower baking quality of two extracted tetraploid wheats compared to their hexaploid parents was accompanied by lowered proportions of residue protein. Mullen and Smith (70) found that a flour with a dough development time of twenty minutes had a much higher proportion of residue protein than a flour with a dough development time of three minutes. Recently Orth and Bushuk (9) showed a strong positive correlation between residue protein content and loaf volume and dough development time in 28 wheat varieties varying in baking quality.

7. Enzymes

A group of flour proteins not mentioned previously is the enzymes. Of special interest to the cereal chemist are the proteases, amylases and oxidases. Low levels of proteases in wheat flours has been implicated

as the cause of "bucky" dough (71). Although earlier workers dismissed the importance of native flour proteases, Miller (72) found that although only one unit of fungal protease activity is added to flour to improve baking quality, each gram of flour already contains approximately 9 units of native protease activity. In general proteases are believed to increase extensibility and improve handling properties of dough.

Several oxidases are now thought to be important in the production of bread. Studies have indicated that lipoxidase could be responsible for a large percentage of total oxygen uptake in dough (73). High levels of lipoxidase in flours therefore could have a maturing effect on the flour proteins as well as the bleaching of flour pigments. Catalase has also been implicated in the bleaching of carotene (74). Kuminori and Mutsumoto (75) found that ascorbic acid, added to flour as an improving agent, is rapidly oxidized to dehydroascorbic acid during dough mixing by ascorbic acid oxidase. They postulated that dehydroascorbic acid, not ascorbic acid, was the active improving (oxidizing) agent.

The amylases are probably the most important group of enzymes in breadmaking. These enzymes hydrolyze damaged starch granules slowly but rapidly hydrolyze starch which has gelatinized during the baking process. Sugars present in the dough as well as maltose released by the former process acts as a food source for yeast resulting in the production of carbon dioxide. Stramberg and Bailey (76) found that the addition of beta amylase to dough did not improve gassing power. Thus the limiting factor in the production of sugars appears to be the rate at

which alpha amylase can produce additional points of attack for beta amylase.

Chromosomal Location and Control of Proteins in Wheat and Related Species

In recent years electrophoretic studies of the proteins of wheat and their related species have been carried out to determine phylogenetic relationships. In a series of papers Johnson and co-workers have confirmed previous cytogenetic evidence (77-80) that the hexaploid bread wheats, Triticum aestivum (AABBDD), are probably derived from the ancient tetraploid, T. dicoccum (AABB) and the wild diploid, Aegilops squarrosa (DD). Electrophoretic patterns of seed extracts showed that nine of the twelve albumin bands present in the hexaploid T. aestivum were homologous with the tetraploid T. dicoccum (81). The three additional bands present in the hexaploid were attributed to the D-genome. In a more recent study Johnson (82) showed that a protein mixture (2:1) of T. dicoccum (AABB) and Ae. squarrosa (DD) gave an electrophoretic pattern almost identical to that of T. aestivum (AABBDD). Electrophoretic studies of isozymes in the Triticum-Aegilops group have shown similar relationships (83,84).

A number of studies have been undertaken to identify the chromosomes in bread wheats that control protein synthesis and baking quality. Although the polyploid nature of bread wheats (AABBDD) have complicated these studies, the development (85) of monosomic stocks for each of the

twenty-one chromosomes of the hexaploid wheat variety, Chinese Spring, has led to the production of aneuploid and substitution lines which allow the effects of single chromosomes to be studied. Recent studies using the above methods have shown that major genes controlling baking quality are located in the B and D genomes. Welsh and Hehn (86) obtained farinograph curves and doughball fermentation times (Pelshenke Test) for the twenty-one monosomic lines of Kharkof MC22, a hard red winter wheat. The presence of chromosome 1D was found necessary to maintain quality. Removal of chromosome 1D resulted in a drastic reduction in the doughball fermentation time and the strength of the dough as measured in the farinograph.

Recent studies with substitution lines have shown that several chromosomes are responsible for quality differences in different varieties of breadwheats. Mixograph and bake tests of substitution lines of Cheyenne into Chinese Spring indicated that chromosomes 1B, 4B, 7B and 5D were mainly responsible for the high baking quality of Cheyenne (87, 88). Welsh et al (89) studied the effects of the complete set of substitution lines in Chinese Spring. Although only one Thatcher chromosome, 2A, caused a significant change in baking quality, eleven Hope and sixteen Timstein chromosomes produced changes. These effects were most pronounced from chromosomes 2B of Hope and 3B and 6B of Timstein.

One limitation of such studies is that the variety, Chinese Spring, has very poor agronomic and flour quality properties. Some of the effects of chromosome substitution may be due to increased protein content rather than increased protein "quality".

Kaltsikes et al (90) have recently studied the AABB tetraploids extracted from three varieties of hard red spring wheat (AABBDD). Farinograph studies showed that the removal of the D genome from the varieties Rescue and Thatcher caused a significant weakening of their doughs. However Tetraprelude had a farinograph curve similar to its parent, Prelude. The retention of quality in the extracted tetraploid was attributed to a translocation involving one of the D-genome chromosomes. Recent cytogenetic evidence supports this conclusion (91).

A number of studies have been concerned with the chromosomal location of genes controlling protein synthesis in bread wheats. Solari and Favret (92,93) analysed the starch gel patterns of gliadins in segregating generations of several crosses of different bread wheat varieties. From these studies it was concluded that genes controlling protein bands tended to be grouped in a few chromosomes. In a later study (94) these workers studied substitution lines of Thatcher into Chinese Spring and found that only two substitution lines involving the 2D and 6D of Thatcher had a large effect on the electrophoretic pattern when compared to disomic Chinese Spring.

Boyd and Lee (95) extracted the gluten proteins from 22 ditelocentric lines of Chinese Spring. Starch gel electrophoresis showed that the removal of the short arm of chromosome 1D resulted in the loss of two slow moving bands. In another study Boyd et al (96) extracted the gluten proteins from Canthatch (AABBDD) and Tetracanthatch (AABB). Tetracanthatch lacked three slow moving bands and one band of intermediate mobility

present in the parent hexaploid. They concluded that these bands were controlled by genes on the D-genome. In the same study synthetic hexaploids produced by crossing Tetracanthatch with several varieties of Ae. squarrosa (DD) contained slow moving gluten bands not present in Tetracanthatch.

Shepherd (97) studied changes in the starch gel patterns of 2M urea extractable protein from the complete set of compensating nullisomic-tetrasomic stocks of Chinese Spring. Also included in these studies were the 21 standard arm ditelocentrics and the 21 tetrasomics of Chinese Spring. Nine of the seventeen major protein bands of Chinese Spring were located on homoeologous group 1 and group 6 chromosomes. Studies with the ditelocentrics indicated that the non-standard arms of group 1 chromosomes and the standard arms of chromosomes 6A and 6D were involved.

Recently Wrigley and Shepherd (98) compared protein maps of disomic Chinese Spring with those of the nulli-tetrasomics by two-dimensional electrophoresis and electrofocusing. Of the 17 major and 29 minor bands present in the disomic, 16 major and 16 minor bands could be assigned to individual chromosomes. All the chromosomes involved in gliadin synthesis appeared to be from homoeologous groups 1 and 6.

Several studies of the effects of the addition of alien chromosomes into bread wheats have been carried out. Shepherd (97) and Shepherd and Jennings (99) analysed the proteins of Holdfast wheat (2n = 42), King

II rye (2n = 14), the Holdfast and King II amphiploid (2n = 56) and seven separate wheat-rye addition lines. The amphiploid contained slow-moving protein bands present in both parents. However in the wheat-rye addition lines only rye chromosome V controlled several slow moving bands which were also present in rye. The other addition lines had electrophoretic patterns similar to the wheat. Thus in rye only one chromosome is responsible for the slow-moving protein bands while in wheat two homoeologous groups (1 and 6) are responsible.

Riley and Ewart (100) used the same material as Shepherd (97) in the study of the effect of adding rye chromosomes to wheat on the amino acid composition. Rye chromosome I increased the lysine content by 8.7% and the cystine content by 10.7%. Other rye chromosomes also had significant effects. Jagannath and Bhatia (101) have recently shown that the substitution of rye chromosome II from Imperial Rye for group 2 chromosomes in Chinese Spring resulted in a significant increase in the protein content of Chinese Spring. No additions or deletions of protein bands were detected by electrophoresis nor was any change in the protein solubility distribution found.

RESULTS AND DISCUSSION

The results and discussion is presented in a series of six papers written in a style suitable for publication in Cereal Chemistry. However references, tables and figures have been numbered consecutively throughout the thesis to avoid confusion. The presentation of the papers follows a logical order based on the development of the project.

Amino Acid Composition and Subunit Structure of Rye Gliadin Proteins Fractionated by Gel Filtration

Abstract

Gliadins of rye (Secale cereale cv. Prolific) were separated by gel filtration on Sephadex G-100 into four distinct groups with apparent molecular weights of greater than 100,000, and of 44,000, 27,000 and 10,000. These fractions contained 48, 29, 7 and 3% by weight of the total protein respectively. Each fraction had a distinct amino acid composition with the two higher molecular weight fractions having high glutamic acid and proline contents while the two lower molecular weight fractions had amino acid compositions resembling albumins. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate before and after reduction was carried out on each fraction to determine subunit structure. The highest molecular weight fraction gave five bands, all with apparent molecular weights between 150,000 and 300,000, but after reduction of disulfide bonds only one band of MW 110,000 was present. The fractions of MW 44,000 and 27,000 from gel filtration each gave a single subunit with MW 42,000. The low molecular weight fraction had a single band of MW 10,000.

Introduction

The important contribution of gliadins to the viscoelastic properties of wheat gluten has prompted a number of studies concerning the separation and characterization of these 70% ethanol soluble proteins. However,

the large number of gliadin components (102) having similar amino acid compositions (29,103) and sequences (58,59,104,105) has made these studies difficult. Rye, (Secale cereale), in contrast to tetra and hexaploid wheats is a diploid and might be expected to have fewer gliadin components. Electrophoretic studies of gliadin proteins from wheat and rye support this conclusion (11,97,106). Available evidence also suggests that the gliadins of wheat and rye are similar in structure. Wheat and rye gliadins have similar electrophoretic mobilities (11,97,106) while immunological (107) and peptide studies (105) suggest similar amino acid sequences. These results are consistent with the hypothesis based on cytogenetic evidence that the rye genome is derived from the same diploid progenitor as are the three genomes of hexaploid wheat (108).

Our studies were initiated due to the lack of data available concerning rye endosperm proteins. In this paper we report studies on the subunit structure and amino acid composition of rye gliadin fractions isolated by gel filtration.

Materials and Methods

Fractionation of Rye Endosperm Proteins. Seed stocks of rye (S. Cereale cv. Prolific) were obtained from the Department of Plant Science, University of Manitoba and milled on a Buhler experimental mill. Duplicate samples (10g) were fractionated into water soluble (albumins), salt soluble (globulins), alcohol soluble (gliadins), acid soluble (glutenins) and insoluble residue proteins by the modified Osborne procedure of Chen and Bushuk (109).

Determination of Protein Content. Protein content was determined by the micro Kjeldahl method using N x 5.7 as the conversion factor.

Gel Filtration of Rye Gliadins. Approximately 250g of Sephadex G-100 (40-120μ) was swollen in excess water and then equilibriated with several changes of eluent (AUC: 0.1M acetic acid, 3M urea, 0.01M cetyltrimethyl ammonium bromide (20)). Dissolved gases were removed by heating the gel in hot water under vacuum. The hot gel slurry was then poured into a K 100/100 column (Pharmacia) and allowed to settle for 12 hours. The gel was packed by downward flow (150 ml/hr) for 12 hours followed by upward flow (140 ml/hr) for 12 hours. The final column volume was 4.5 liters.

Protein samples of approximately 1000 mg were dissolved in 50 ml of AUC and eluted by upward flow at rates of 140 ml/hr. The effluent was monitored at 280 nm with an Isco model UA_2 ultraviolet analyser and appropriate peaks were collected. Each fraction was then dialized for 5 days against water and lyophilized. Molecular weights were estimated by calibration of the column with proteins of known molecular weights[a] as recommended by Whitaker (110). Recoveries of nitrogen from the column ranged from 80% to 85%. Each fraction was rechromatographed on a 2.5 x 40 cm column of Sephadex G-100 in AUC.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. SDS electrophoresis was performed in a 5% polyacrylamide gel with pH 7.3 phosphate buffer containing 0.1% SDS according to the method of Orth and Bushuk (111)[a]. Gels were stained in Coomassie Brilliant Blue R250

[a]Molecular weight curves obtained with proteins of known molecular weight are shown in the Appendix.

according to the method of Koenig et al (112).

Amino Acid Analysis. Amino acid analysis was carried out on a Beckman Model 121 amino acid analyzer by the method of Spackmann et al (113). Protein samples (8mg) were dissolved in 4ml of triple distilled 6N hydrochloric acid, frozen and evacuated. Hydrolysis was carried out for 24 hours at 110°C. The hydrolysates were dried under vacuum over sodium hydroxide and then dissolved in pH 2.2 citrate buffer (0.1M).

Results and Discussion

Solubility Distribution of Rye Endosperm Proteins. The distribution on the basis of solubility of the rye endosperm proteins was similar to those reported by Chen and Bushuk (109). The Osborne fractions of rye endosperm contained 29, 8, 17, 15 and 31% of the total protein as water, salt, alcohol, acid soluble and residue proteins respectively. Wheat endosperm proteins have been shown to yield in much lower proportions of water soluble proteins and a much higher proportion of alcohol soluble proteins (114).

Amino Acid Composition of Rye Endosperm Solubility Classes. The amino acid compositions of proteins in the different solubility classes of the rye showed major differences (Table 2). The albumins and globulins contained more ionic amino acids (basic and acidic) and less polar amino acids than the gliadins and glutenins. Each solubility class could be identified on the basis of the content of six amino acids: lysine, arginine, glutamic acid, proline, glycine and alanine. For example,

TABLE 2. Amino Acid Composition of Rye (cv. Prolific) Endosperm proteins (mole %)*

Amino Acid	Albumins	Globulins	Gliadins	Glutenins
Lysine	2.1	4.3	0.7	2.0
Histidine	1.5	2.1	1.3	1.3
Ammonia	25.7	12.7	37.3	31.5
Arginine	2.6	4.8	1.5	1.7
Aspartic acid	4.1	7.5	2.1	2.7
Threonine	3.2	4.0	2.0	2.5
Serine	5.1	5.5	4.9	5.3
Glutamic acid	27.7	16.4	36.7	33.7
Proline	16.6	6.5	20.3	16.6
Glycine	4.3	8.1	2.3	7.2
Alanine	4.5	7.4	2.5	3.4
Valine	10.5	14.0	9.6	9.4
Methionine	1.3	1.7	1.1	1.0
Isoleucine	3.5	3.9	3.2	2.3
Leucine	6.6	7.6	5.9	5.2
Tyrosine	1.6	2.2	1.0	2.3
Phenylalanine	4.6	3.5	4.6	3.4

*Cystine and tryptophan not determined

gliadins could be distinguished from glutenins by their lower content of lysine, glycine and alanine and higher content of glutamic acid and proline. The amino acid composition of the various rye protein fractions were similar to published data for wheat proteins (48).

Gel Filtration of Rye Gliadins. The elution profile obtained by chromatography on Sephadex G-100 of the gliadin proteins of rye is shown in Figure 1. Five fractions were isolated with average molecular weights of greater than 100,000 (F_1), and of 76,000 (F_2), 44,000 (F_3), 27,000 (F_4) and 10,000 (F_5). Of the five fractions, F_1 (48%) and F_3 (29%) accounted for over 75% by weight of the total gliadin protein while fractions F_2, F_4 and F_5 accounted for 14%, 7% and 3% respectively. Rechromatography of fractions F_1, F_3, F_4 and F_5 gave single peaks with values of Ve/Vo (Ve = elution volume and Vo = void volume) identical to the corresponding original fraction. Rechromatography of F_2 yielded two peaks with values of Ve/Vo identical to F_1 and F_3.

Amino Acid Composition of Rye Gliadin Fractions. The amino acid composition of the five gliadin fractions and of whole gliadin are shown in Table 3. The high molecular weight fraction (F_1) had very high contents of proline and glutamic acid while the third fraction (F_3) had a similar high proline content but less glutamic acid. The low molecular weight fractions (F_4 and F_5) had amino acid compositions similar to the albumin fraction. There was a definite trend toward an increase in proline and glutamic acid and a decrease in the basic amino acids, aspartic acid and glycine as molecular weight increased.

Fig. 1. Sephadex G-100 chromatography of rye (cv. Prolific) gliadins in AUC.

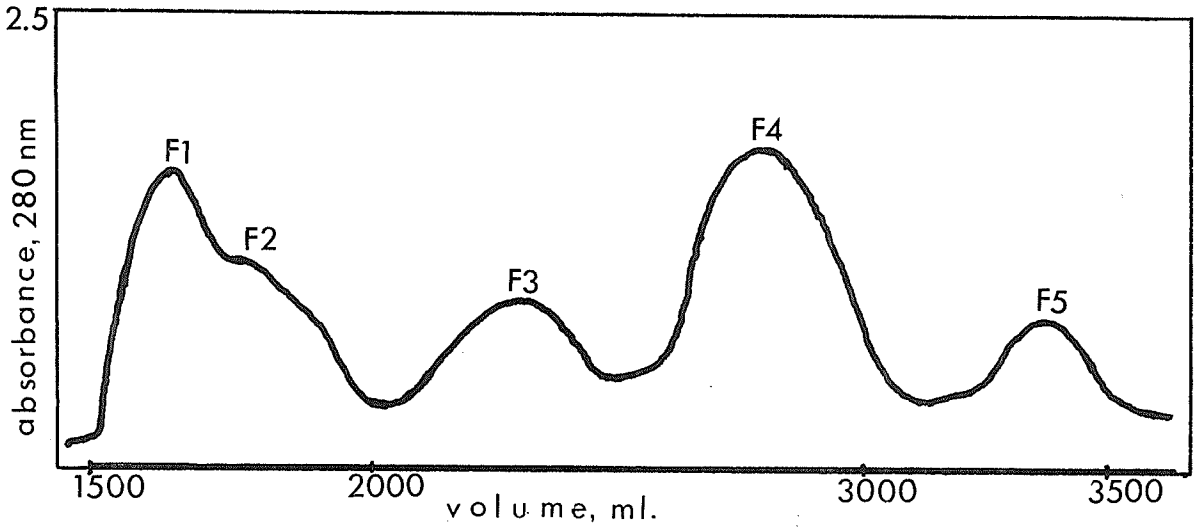

2.5
absorbance, 280 nm
F1
F2
F3
F4
F5
1500
2000
3000
3500
volume, ml.

TABLE 3. Amino Acid Composition of Rye (cv. Prolific) Gliadin Fractions from Sephadex G-100 Chromatography in AUC (mole %)*

Amino Acid	F_1	F_2	F_3	F_4	F_5
Lysine	0.5	0.5	0.7	2.2	2.8
Histidine	1.1	1.1	1.2	1.6	1.7
Arginine	0.8	0.9	1.4	2.1	2.6
Aspartic Acid	1.4	1.4	2.3	3.8	5.1
Threonine	1.4	1.4	2.3	3.8	3.8
Serine	4.7	4.4	4.5	5.5	7.6
Glutamic Acid	41.4	42.7	35.9	25.3	24.6
Proline	20.8	21.1	20.0	14.4	11.2
Glycine	1.5	1.6	2.3	3.8	8.4
Alanine	2.0	1.9	2.1	3.6	4.7
Valine	10.1	9.3	4.7	13.2	10.0
Methionine	0.8	0.7	1.1	1.4	1.5
Isoleucine	2 3	2.5	4.0	5.3	3.9
Leucine	4.6	4.5	6.2	7.7	6.6
Tyrosine	1.0	0.9	0.9	1.3	2.0
Phenylalanine	4.6	4.9	5.1	4.4	3.5

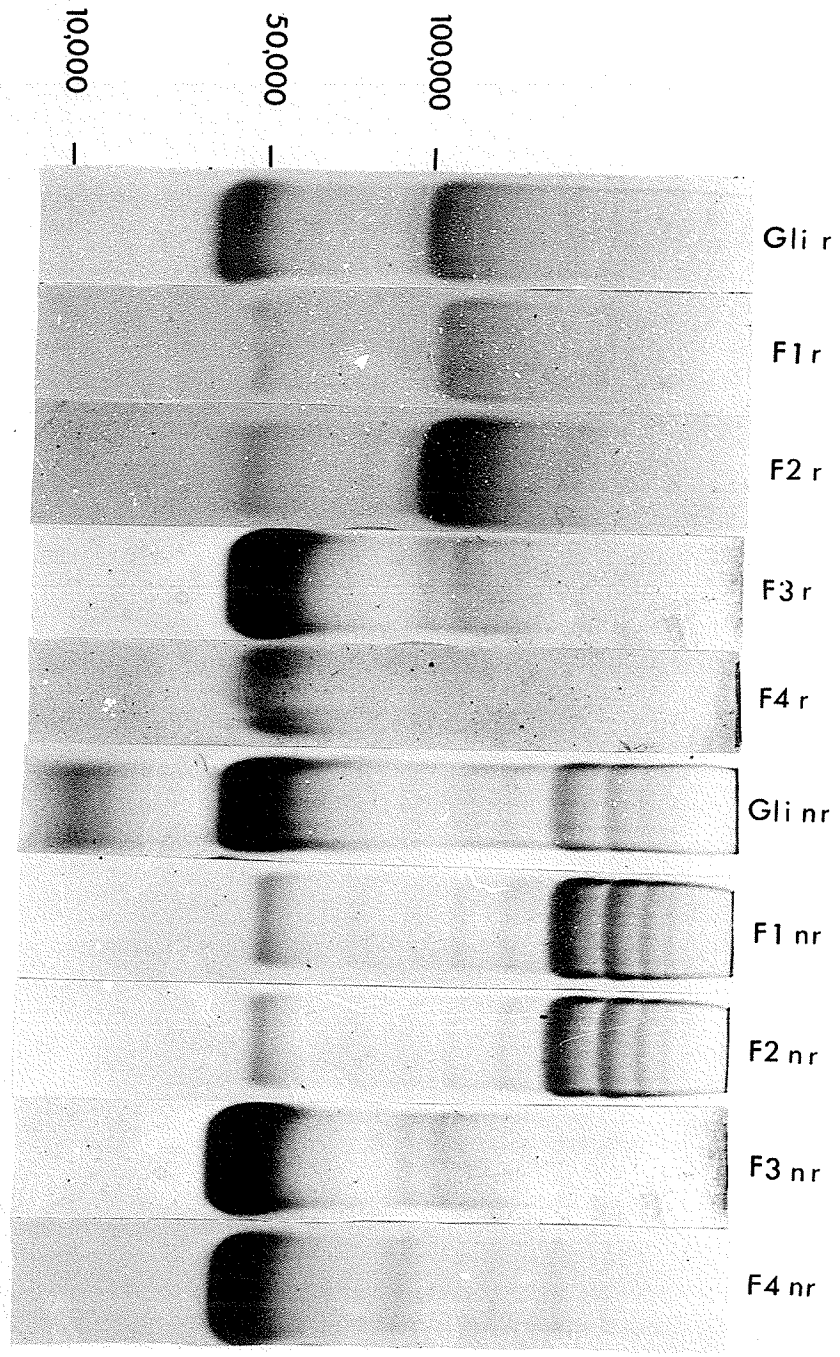
*Cystine and tryptophan not determined

The unique amino acid composition of the high molecular weight fraction indicates that these proteins are not produced through inter-chain disulfide bonding of the lower molecular weight gliadins in fractions F_3, F_4 or F_5. Neither can this high molecular weight gliadin fraction be considered to be glutenin-like since glutenins generally contain higher relative proportions of glycine and lower contents of proline and glutamic acid (48,115).

SDS-Polyacrylamide Gel Electrophoresis of Rye Gliadin Fractions. In order to determine the subunit structure of the isolated rye gliadin fractions, they were subjected to electrophoresis on polyacrylamide gel in the presence of SDS. The lower molecular weight fractions (F_3 and F_4) gave similar patterns for both the reduced and non-reduced preparations (Fig. 2), indicating that these proteins are single chain polypeptides with no inter-chain disulfide bonding. For both gliadin fractions only one band was present. In each case a molecular weight of approximately 42,000 was calculated.

Although the apparent molecular weight of fractions F_3 and F_4 subunits were identical, these fractions differed widely in amino acid composition and therefore do not represent similar polypeptide chains. The molecular weight of fraction F_3 gliadins determined by SDS electrophoresis agreed with the values obtained by gel filtration (MW 44,000). However the molecular weight of fraction F_4 gliadins were much lower by gel filtration (MW 27,000) than by SDS-gel electrophoresis (MW = 42,000). There is no obvious reason for the discrepancy in the two molecular weight values determined for F_4.

Fig. 2. SDS-PAGE patterns of reduced (r) and nonreduced (nr) rye (cv. Prolific) gliadin fractions from Sephadex G-100 chromatography in AUC.


10,000
50,000
100,000
Gli r
F1 r
F2 r
F3 r
F4 r
Gli nr
F1 nr
F2 nr
F3 nr
F4 nr

The SDS-gel pattern for the non-reduced high molecular weight rye gliadin fraction (F_1) is shown in Figure 2. At least five major bands could be identified in the high molecular weight region (MW = 150,000-300,000) of the gel. After reduction of disulfide bands with β-mercaptoethanol, the high molecular weight gliadin fraction gave a single band with a molecular weight of approximately 110,000. The multiplicity of high molecular weight bands in the non-reduced pattern may arise in two ways. The high molecular weight gliadins in rye may be produced through inter-chain disulfide bonding between polypeptide chains with MW's of approximately 110,000. Alternately there may be conformational isomers due to different sites of disulfide bonding within the same polypeptide chains which may determine the mobility of the SDS-protein complex. It has been shown that intact disulfide bonds reduce SDS binding to proteins and thus decrease the mobility of the complex (116). A faint band (MW = 44,000) was also detected in the high molecular weight fraction but was not detectable following rechromatography.

General Discussion

Gel filtration in a strongly dissociating solvent (AUC) separated the gliadin proteins of rye into four distinct fractions with molecular weights varying from 10,000 to over 100,000. Each fraction had a distinct amino acid composition which eliminated the possibility that the high molecular weight fractions were the result of association or inter-chain disulfide bonding of the lower molecular weight fractions.

The subunit structure of each fraction was determined by SDS-polyacrylamide gel electrophoresis after reduction with β-mercaptoethanol.

The high molecular weight gliadin fraction had a single subunit of MW 110,000 while both fractions F_3 and F_4 had single subunits of MW 42,000. The whole gliadin gave an additional subunit of MW 10,000 which probably represents fraction F_5. It is probable that the calculated subunit molecular weights are high due to the high proline content which causes a more rigid polypeptide-SDS complex, slowing the rate of migration in the gel (117).

SDS-polyacrylamide gel electrophoretic studies by Bietz and Wall (44) have shown that the major gliadin subunits of a HRW wheat have MW's of 11,400, 36,500, 44,200, 69,300 and 78,100. The high molecular weight wheat gliadins had subunits of MW's 44,200 and 36,500. Thus in wheat, high molecular weight gliadins which constitute less than 10% of the total gliadins are composed of several much smaller subunits. The high molecular weight rye gliadins which account for 60% of the total gliadins do not appear to have any subunits less than MW 100,000. The high molecular weight subunits may also lack the ability to form inter-chain disulfind bonds as do the lower molecular weight subunits.

Amino Acid Composition and Subunit Structure of Wheat Gliadin Fractions Separated by Gel Filtration

Abstract

Gliadins, prepared by Osborne fractionation, from four wheat varieties representing three different classes were fractionated on Sephadex G-100 in a dissociating solvent. Five fractions of average molecular weights greater than 100,000 and of 85,000, 44,000, 27,000 and 10,000 were obtained from each variety. Significant varietal variation in the molecular weight distribution was found. The first four fractions of each variety had amino acid compositions similar to whole gliadin but the fifth fraction had an amino acid content similar to wheat albumin. SDS-polyacrylamide gel electrophoresis showed that the hexaploid varieties had identical subunits with major subunits of MW's 10,000, 36,000, 40,000 and 50,000 and minor subunits of MW's 53,000, 78,000, 82,000, 88,000, 108,000, 120,000 and 130,000. The tetraploid durum variety lacked minor subunits of 88,000, 120,000 and 130,000. Comparison of reduced and non-reduced fractions indicated that the lower molecular weight fractions were single chain proteins. The non-reduced high molecular weight fractions gave a large number of bands of apparent MW 150,000-300,000 but upon reduction gave three major subunits of MW's 40,000, 50,000 and 53,-000.

Introduction

The gliadin proteins of wheat flour have been shown to consist of a large number of different sized components ranging in molecular weight

from 10,000 to over 100,000. They are mainly single polypeptide chains stabilized by intra-chain disulfide bonding (20,40,43,44,52,61). Bietz and Wall (44) have shown by electrophoresis in the presence of sodium dodecyl sulfate (SDS) that reduced gliadins contain polypeptide chains with MW's of 11,400, 36,500, 44,200, 69,300 and 78,100. In contrast to the wide variation in molecular weights, various gliadin components have similar amino acid compositions and sequences (29,58,59,103-105).

Recently we have shown (119) that alcohol soluble (gliadins) proteins of rye can be fractionated by gel filtration into four fractions varying in molecular weight. Each fraction had a distinct amino acid composition and subunit structure. In the present paper we have extended this study to the alcohol soluble (gliadins) proteins isolated from different classes of wheat. Quantitative differences in gliadin fractions isolated by gel filtration as well as the amino acid composition and subunit structure of each fraction has been studied.

Materials and Methods

Fractionation of Wheat Endosperm Proteins. Four varieties of wheat were obtained from stocks available in the Department of Plant Science, University of Manitoba: Manitou (HRS), Pembina (HRS), Talbot (SWW) and Stewart 63 (amber durum). The wheats were milled on a Buhler experimental mill and the resulting flours were fractionated into water soluble (albumins), salt soluble (globulins), 70% ethanol soluble (gliadins), acid soluble (glutenins) and insoluble residue by the modified Osborne procedure of Chen and Bushuk (109).

Gel Filtration of Gliadins. The wheat gliadins were fractionated on a K 100/100 column (Pharmacia) containing approximately 4.5 liters of Sephadex G-100 swollen with the AUC solvent of Meredith and Wren (20) as previously described (119). Protein (N x 5.7) was determined by the micro-Kjeldahl procedure.

SDS-Polyacrylamide Gel Electrophoresis. SDS-gel electrophoresis was performed in a 5% polyacrylamide gel with pH 7.3 phosphate buffer containing 0.1% SDS according to the method of Orth and Bushuk (111). Gels were stained in Coomassie Brilliant Blue R250 by the method of Koenig et al (112).

Amino Acid Analysis. Amino acid analysis was carried out as previously described (119).

Results and Discussion

Gel Filtration of Wheat Gliadins. Gel filtration profiles of each variety of wheat gliadin obtained by chromatography in the strongly dissociating solvent AUC are shown in Figure 3. Five gliadin fractions were isolated from each variety with approximate molecular weights of greater than 100,000 (F_1), 85,000 (F_2), 44,000 (F_3), 27,000 (F_4) and 10,000 (F_5). There was little variation in calculated molecular weight between corresponding fractions of different varieties. Rechromatography of the second fraction (MW approximately 85,000) gave two peaks with values of Ve/Vo identical to the first and third fraction. Rechromatography of the other four fractions gave single peaks with values of Ve/Vo identical to the

Fig. 3. Sephadex G-100 chromatography of wheat gliadins in AUC.

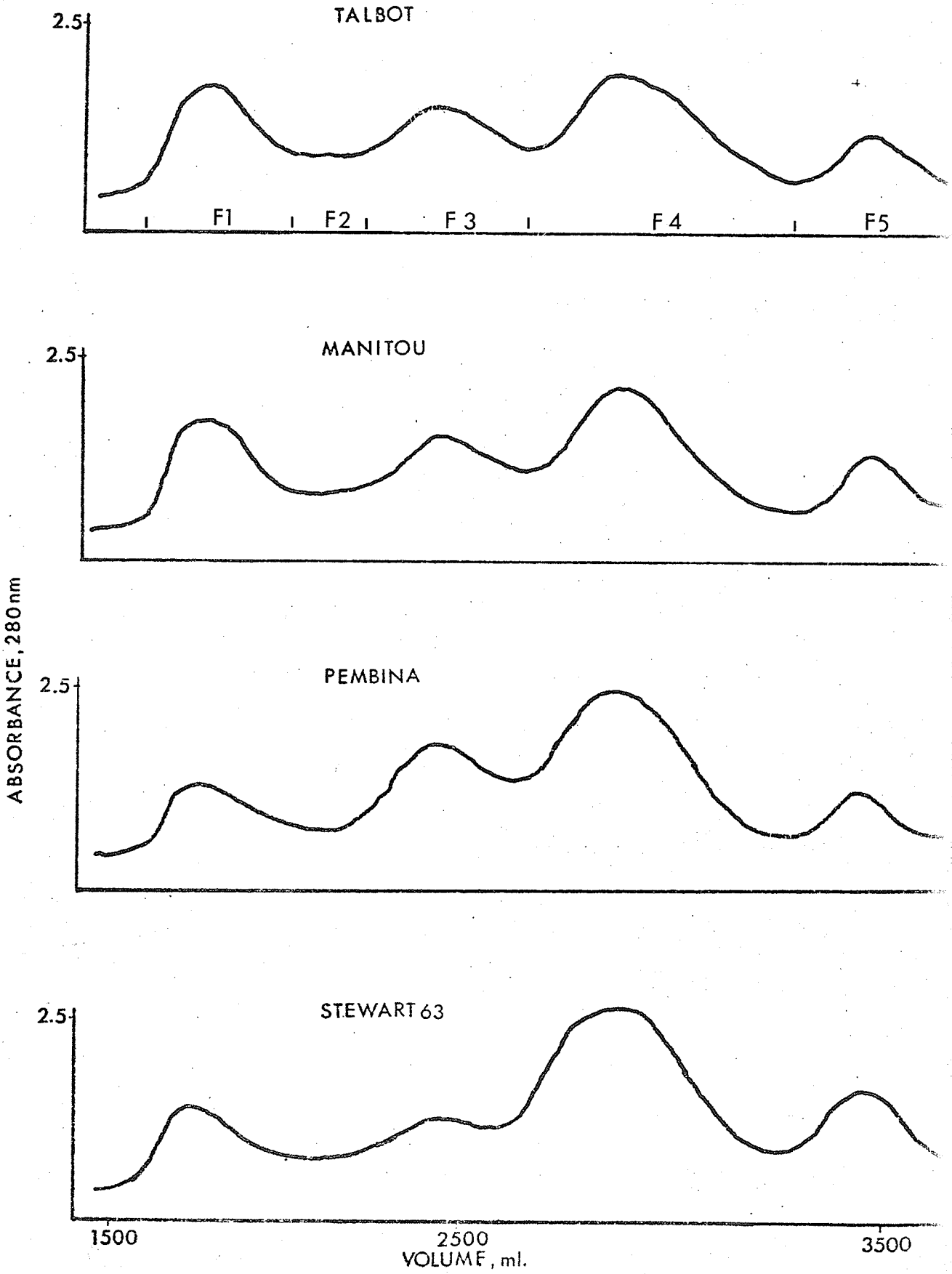

TALBOT
2.5
F1
F2
F3
F4
F5
MANITOU
2.5
PEMBINA
2.5
STEWART 63
2.5
ABSORBANCE, 280nm
1500
2500
3500
VOLUME, ml.

Table 4. Distribution of wheat gliadin fractions from Sephadex G-100*

Variety	Class	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5
Pembina	HRW	11	6	27	51	5
Manitou	HRW	26	11	17	41	5
Talbot	SWW	32	11	15	33	9
Stewart 63	Durum	8	4	6	63	19

*Data given represents % weight of recovered nitrogen

corresponding original fraction.

The weight distribution of the gliadin fractions isolated by gel filtration are shown in Table 4. Recoveries of protein from the column ranged from 75 to 85%. Varietal differences in the distribution of the gliadin fractions were apparent. Stewart 63 and Pembina had low relative contents of high molecular weight gliadins (F_1 and F_2) while Talbot and Manitou had substantially larger amounts. The proportion of fraction 3 gliadins in the four wheat varieties varied from 6 to 27% with Stewart 63 having the lowest and Pembina the highest. The highest relative proportion of gliadins in all varieties occurred in the fourth fraction. Stewart 63 had the highest content (63%) while the SWW variety, Talbot, had the lowest (33%). Stewart 63 also had the highest proportion of low molecular weight gliadins.

Fractionation and reconstitution techniques have shown that the gliadin proteins in wheat flour are important in the control of loaf volume potential (50,120). However attempts to correlate qualitative electrophoretic differences with loaf volume potential have been unsuccessful (9,45,121). The present results show that significant differences in the molecular weight distribution of gliadins exist in different varieties of wheat. Gliadin proteins varying in molecular weight might be expected to impart different properties to the hydrated flour complex.

Amino Acid Composition of Wheat Gliadin Fractions. The amino acid composition of the gliadins of the four wheat varieties are shown in Table 5. The gliadins had characteristically low contents of basic amino

Table 5. Amino acid compositions of gliadins (mole %)*

Amino acid	Pembina	Manitou	Talbot	Stewart 63
Lysine	0.5	0.6	0.7	0.5
Histidine	1.6	1.4	1.4	1.6
Ammonia	39.1	40.0	39.8	40.3
Arginine	1.5	1.3	1.6	1.6
Aspartic acid	2.5	2.5	2.6	2.7
Threonine	2.1	2.0	2.2	1.9
Serine	5.5	4.7	5.3	4.7
Glutamic acid	40.0	40.0	38.8	39.7
Proline	17.5	18.5	17.5	17.6
Glycine	2.9	2.6	3.3	2.6
Alanine	2.9	2.7	3.0	3.0
Valine	4.0	4.0	4.2	4.1
Methionine	1.1	0.9	1.2	1.2
Isoleucine	3.8	3.9	3.8	4.1
Leucine	7.2	7.0	7.4	7.3
Tyrosine	1.9	1.7	2.1	2.1
Phenylalanine	4.9	5.3	4.8	4.9

*Tryptophan and Cystine not determined

Table 6. Amino acid composition of gliadin fractions (mole %)*

Amino acid	Fraction 1			Fraction 3			Fraction 4		
	M[a]	T[a]	S[a]	M	T	S	M	T	S
Lysine	0.5	0.7	0.7	0.5	0.7	0.5	0.5	0.5	0.4
Histidine	1.6	1.7	1.4	1.2	1.2	1.4	1.5	1.7	1.4
Arginine	1.8	2.0	1.5	1.1	1.3	1.2	1.5	1.5	1.4
Aspartic acid	1.7	1.6	1.7	1.8	1.9	1.3	3.0	2.7	2.6
Threonine	2.8	2.7	2.6	1.9	2.2	2.0	1.8	1.8	1.9
Serine	6.3	6.6	6.9	4.5	5.1	4.7	4.8	4.9	4.6
Glutamic acid	39.1	38.2	38.8	42.6	39.9	42.7	39.5	39.4	44.4
Proline	15.6	16.4	17.0	20.1	19.4	23.0	17.6	18.0	17.3
Glycine	4.5	4.9	4.2	2.4	3.0	2.4	2.5	2.7	2.3
Alanine	2.4	2.6	2.5	2.3	2.7	1.8	2.9	3.1	2.6
Valine	4.3	4.0	4.0	3.2	3.6	2.6	4.3	4.2	3.8
Methionine	1.2	1.4	1.4	0.7	1.1	0.6	0.5	1.1	0.8
Isoleucine	4.0	3.3	3.7	3.6	3.3	3.4	4.3	3.8	3.8
Leucine	7.3	7.3	7.0	6.2	6.8	5.9	7.1	7.5	6.3
Tyrosine	1.6	1.9	1.1	0.6	0.9	0.6	1.8	2.4	1.8
Phenylalanine	4.2	4.7	4.5	6.0	5.8	5.9	4.9	4.7	4.6

* Tryptophan and cystine not determined

[a] M, T and S refer to Manitou, Talbot and Stewart 63, respectively

Table 7. Amino acid composition of the low molecular weight gliadin fraction (mole %)*

Amino acid	Pembina	Manitou	Talbot	Stewart 63
Lysine	3.6	2.6	0.9	1.6
Histidine	1.5	1.6	1.6	1.6
Arginine	1.4	1.8	1.7	2.3
Aspartic acid	4.8	4.8	3.2	4.8
Threonine	3.6	3.7	2.1	3.4
Serine	7.4	8.4	5.3	5.3
Glutamic acid	28.2	31.2	38.0	31.4
Proline	14.4	12.5	17.0	14.2
Glycine	6.5	6.9	3.1	4.7
Alanine	4.6	5.3	3.5	6.0
Valine	4.0	4.4	4.2	5.1
Methionine	1.1	0.9	1.1	1.2
Isoleucine	3.6	4.3	3.8	4.2
Leucine	8.1	6.9	7.5	7.0
Tyrosine	2.1	1.9	2.3	2.5
Phenylalanine	5.1	2.8	4.6	3.8

*Tryptophan and cystine not determined

acids and high contents of glutamic acid and proline. The high content of ammonia in the hydrolysates indicated that most of the glutamic acid present was in the form of glutamine. There was little varietal variation in amino acid composition between varieties. Even the absence of the D-genome in the durum variety, Stewart 63, did not have any effect on the amino acid composition. This result is in agreement with Dronzek et al (48) who found that the gliadins of an extracted tetraploid (AABB) had a similar amino acid composition when compared to its parent hexaploid (AABBDD).

The first four gliadin fractions (F_{1-4}) from each variety had amino acid compositions similar to whole gliadin (Table 6). Results for fraction 2 gliadins and for Pembina are omitted in the table. The Pembina fractions had amino acid compositions almost identical to those of Manitou. Differences between equivalent fractions among the four varieties were small. Within each variety, the amino acid composition of the first four gliadin fractions were similar. Fraction 1 gliadins tended to have more serine and glycine and less proline than the other fractions. Fraction 3 gliadins had more proline and phenylalanine and less leucine and tyrosine while fraction 4 gliadins had more aspartic acid.

In contrast to the first four gliadin fractions, the low molecular weight fraction (F_5) of each variety except Talbot had amino acid compositions more closely related to albumin (Table 7). The content of glutamic acid and proline was much lower in this fraction than in whole gliadin. Contents of lysine, aspartic acid, threonine, glycine and alanine were higher. The low molecular weight gliadin fraction of the SWW variety,

Talbot, had an amino acid composition similar to whole gliadin.

SDS-Polyacrylamide Gel Electrophoresis of Wheat Gliadin Fractions. The wheat gliadin fractions isolated by gel filtration were characterized by SDS-gel electrophoresis before and after reduction of their disulfide bonds with β-mercaptoethanol. Each sample was run at two protein concentrations. At high protein concentration minor bands could be characterized but major bands were not well resolved due to their intense staining. At lower protein concentrations major bands were well resolved but minor bands were not visible. The SDS-gel pattern of whole gliadins from each variety at high protein concentration are shown in Figure 4. The molecular weights determined for the subunits of whole gliadin and the gliadin fractions are shown in Table 8.

The three hexaploid wheat varieties all showed identical gliadin subunits after reduction. Major subunits had calculated MW's of 10,000, 36,000, 40,000 and 50,000. Minor subunits had MW's of 53,000, 78,000, 82,000, 88,000, 108,000, 120,000 and 130,000. The tetraploid durum variety Stewart 63, had major subunits identical to the hexaploid varieties but lacked minor subunits of MW's 88,000, 120,000 and 130,000. The SDS-gel pattern of the non-reduced gliadins from both the hexaploid and tetraploid varieties had a large number of bands in the high molecular weight region (MW 150-500,000) of the gel. Major bands also occurred in the lower molecular weight region of the gel.

The SDS-gel patterns of the non-reduced high molecular weight gliadin fractions (F_1) of each variety had a large number of bands in the high

molecular weight region of the gel (MW approximately 150,000-300,000) (Fig. 5). Upon reduction of disulfide bonds, both the hexaploid and tetraploid varieties gave major subunits of MW's 40,000 and 50,000 and minor subunits of 53,000 and 108,000. The hexaploid varieties had additional minor subunits ot MW's 120,000 and 130,000. From this data it appears that the high molecular weight wheat gliadins are built up mainly of smaller polypeptide chains of MW's 40,000, 50,000 and 53,000 through inter-chain disulfide bonds. The subunits of the high molecular weight gliadins found in these varieties are higher than the values of 36,500 and 44,200 reported by Bietz and Wall (44) for HRS wheats. However these differences are probably due to inherent errors in the calculation of molecular weights by this method (122) rather than representing varietal differences.

As mentioned earlier, the fraction 2 gliadins when rechromatographed gave two peaks with values of Ve/Vo corresponding to fraction 1 and fraction 3 gliadins. The SDS-gel patterns of this fraction for each variety gave patterns similar to fraction 1 gliadins (Fig. 6).

The fraction 3 gliadins for both the hexaploid and tetraploid varieties gave major subunits of MW's 40,000 and 50,000 (Fig. 7). The fraction 4 gliadins had major subunits of MW's 36,000 and 40,000 (Fig. 8). The non-reduced fraction 3 and fraction 4 gliadins had bands with calculated molecular weights identical to the corresponding reduced fractions. Thus both these fractions contain proteins that are single chain polypeptides. Fraction 3 gliadins from both the hexaploid and

Table 8. Molecular weights of gliadin subunits determined by SDS-gel electrophoresis (MW x 10^3).

Gliadin Fraction	Major Subunits		Minor Subunits	
	Hexaploid	Tetraploid	Hexaploid	Tetraploid
Whole gliadin	10, 36, 40, 50	10, 36, 40, 50	53, 78, 82 88, 108, 120, 130	53, 108, 78, 82
Fraction 1	40, 50	40, 50	53, 108, 120, 130	53, 108
Fraction 2	40, 50	40, 50	53, 78, 82, 88, 108, 120, 130	53, 78, 82, 108
Fraction 3	40, 50	40, 50	78, 82, 88	78, 82
Fraction 4	36, 40	36, 40		
Fraction 5	10	10		

Fig. 4. SDS-PAGE patterns of reduced (r) and non reduced (nr) wheat gliadins of Talbot (T), Manitou (M), Pembina (P) and Stewart 63 (S).

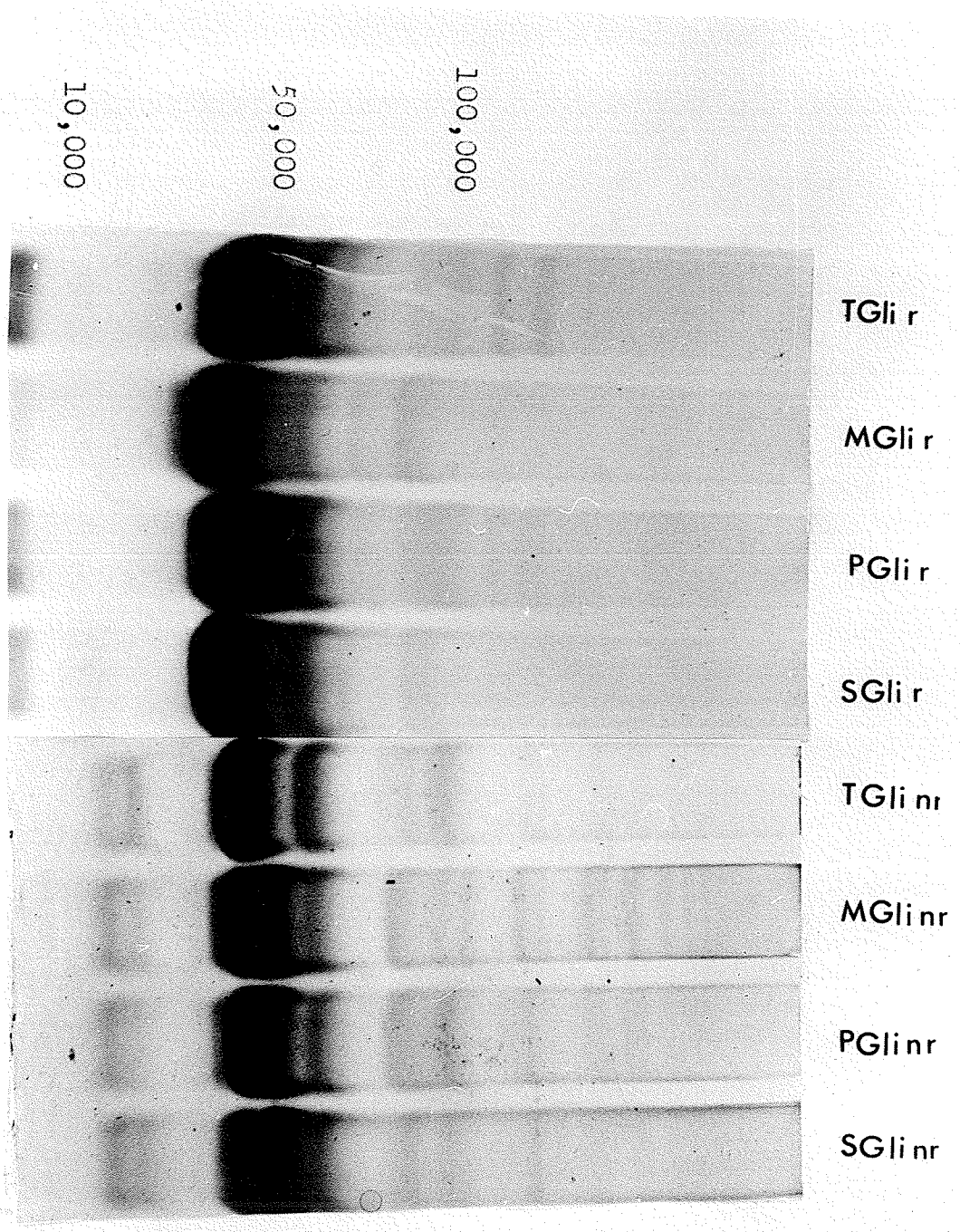

10,000
50,000
100,000
TGli r
MGli r
PGli r
SGli r
TGli nr
MGli nr
PGli nr
SGli nr

Fig. 5. SDS-PAGE patterns of reduced (r) and nonreduced (nr) high molecular weight gliadin fractions (F_1) of Talbot (T), Manitou (M), Pembina (P) and Stewart 63 (S) from gel filtration.

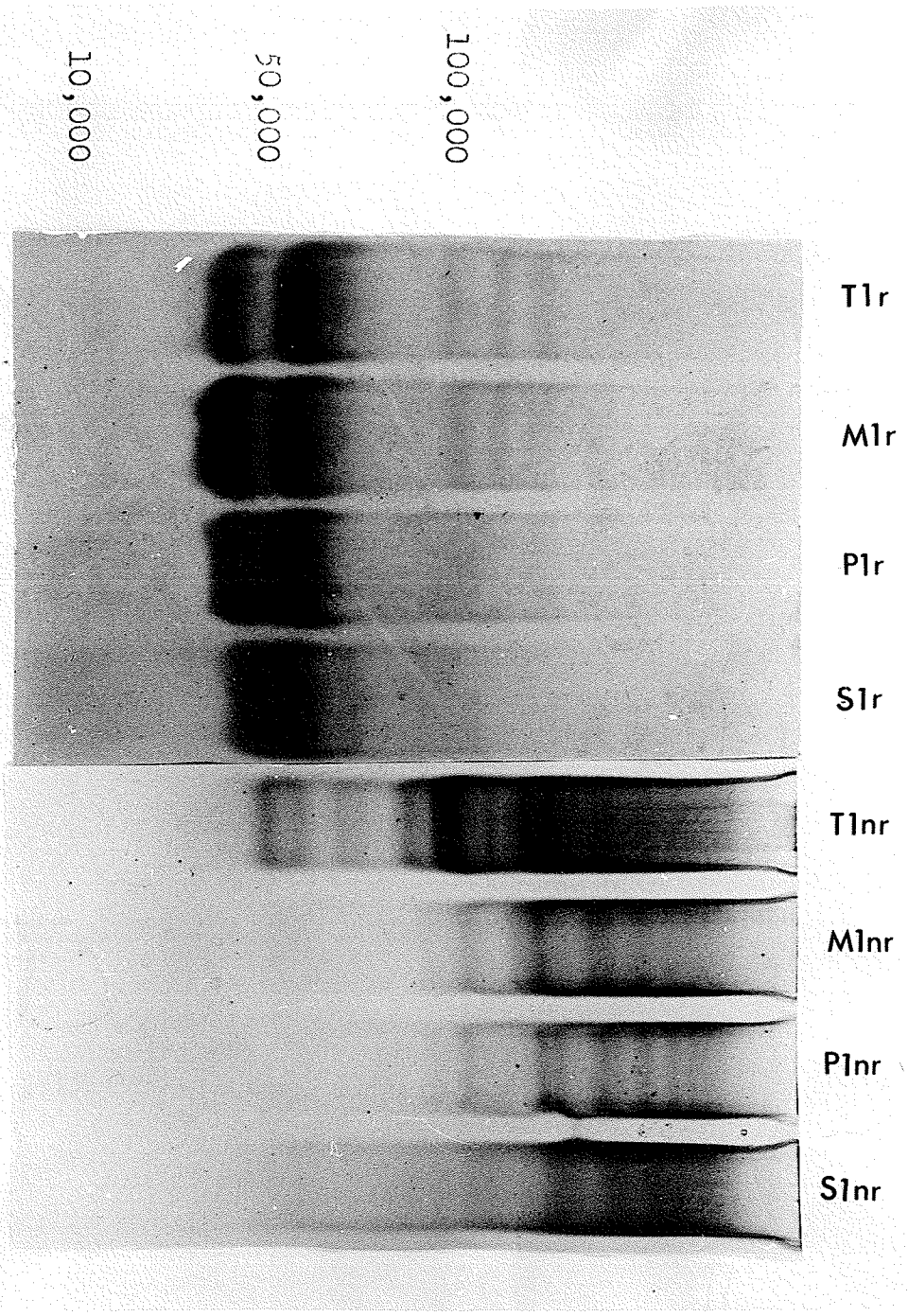

10,000
50,000
100,000
T1r
M1r
P1r
S1r
T1nr
M1nr
P1nr
S1nr

Fig. 6. SDS-PAGE patterns of reduced (r) and non reduced (nr) fraction 2 gliadins of Talbot (T), Manitou (M), Pembina (P) and Stewart 63 (S) from gel filtration.

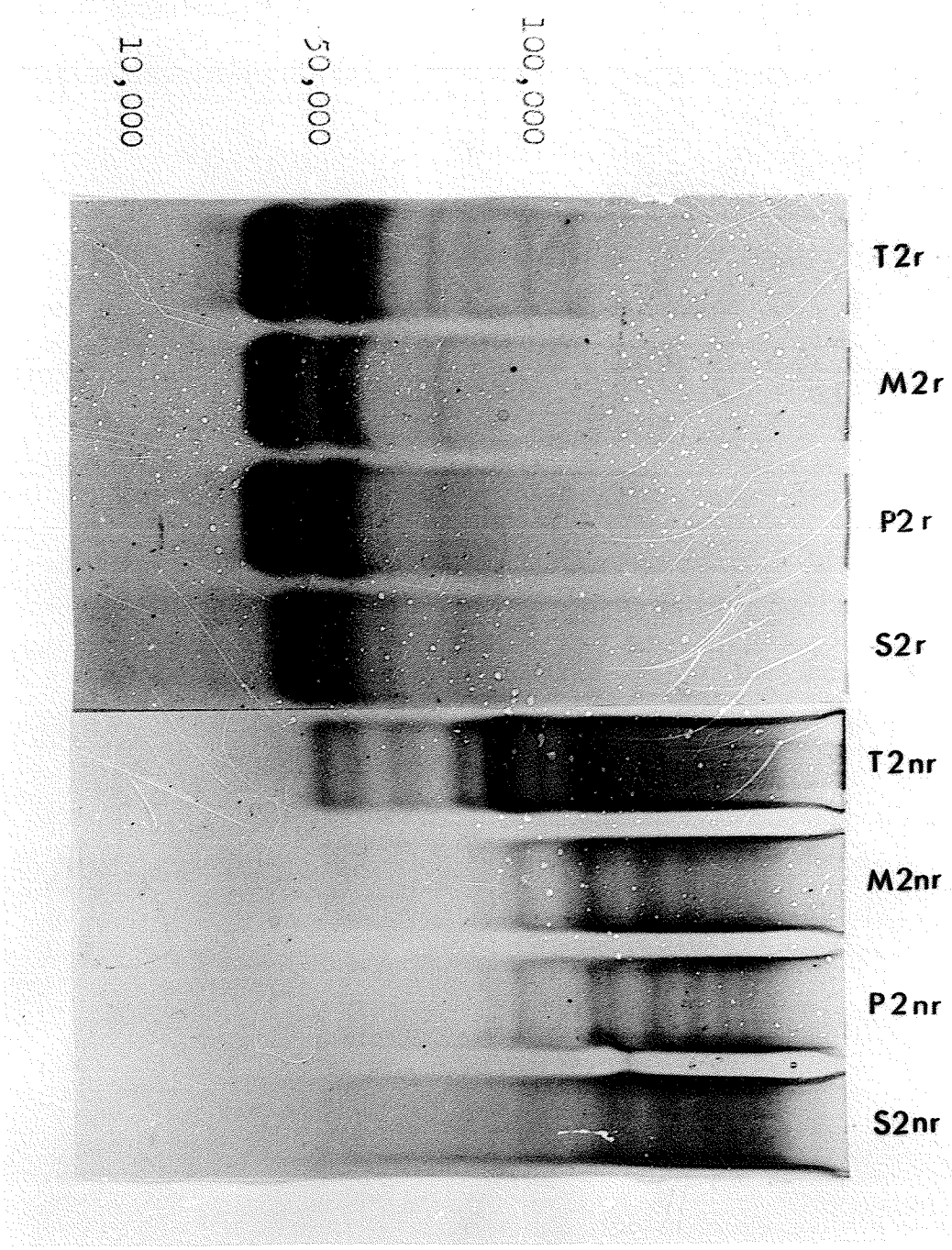

10,000
50,000
100,000
T2r
M2r
P2r
S2r
T2nr
M2nr
P2nr
S2nr

Fig. 7. SDS-PAGE patterns of reduced (r) and non reduced (nr) fraction 3 gliadins of Talbot (T), Manitou (M), Pembina (P) and Stewart 63 (S) from gel filtration.

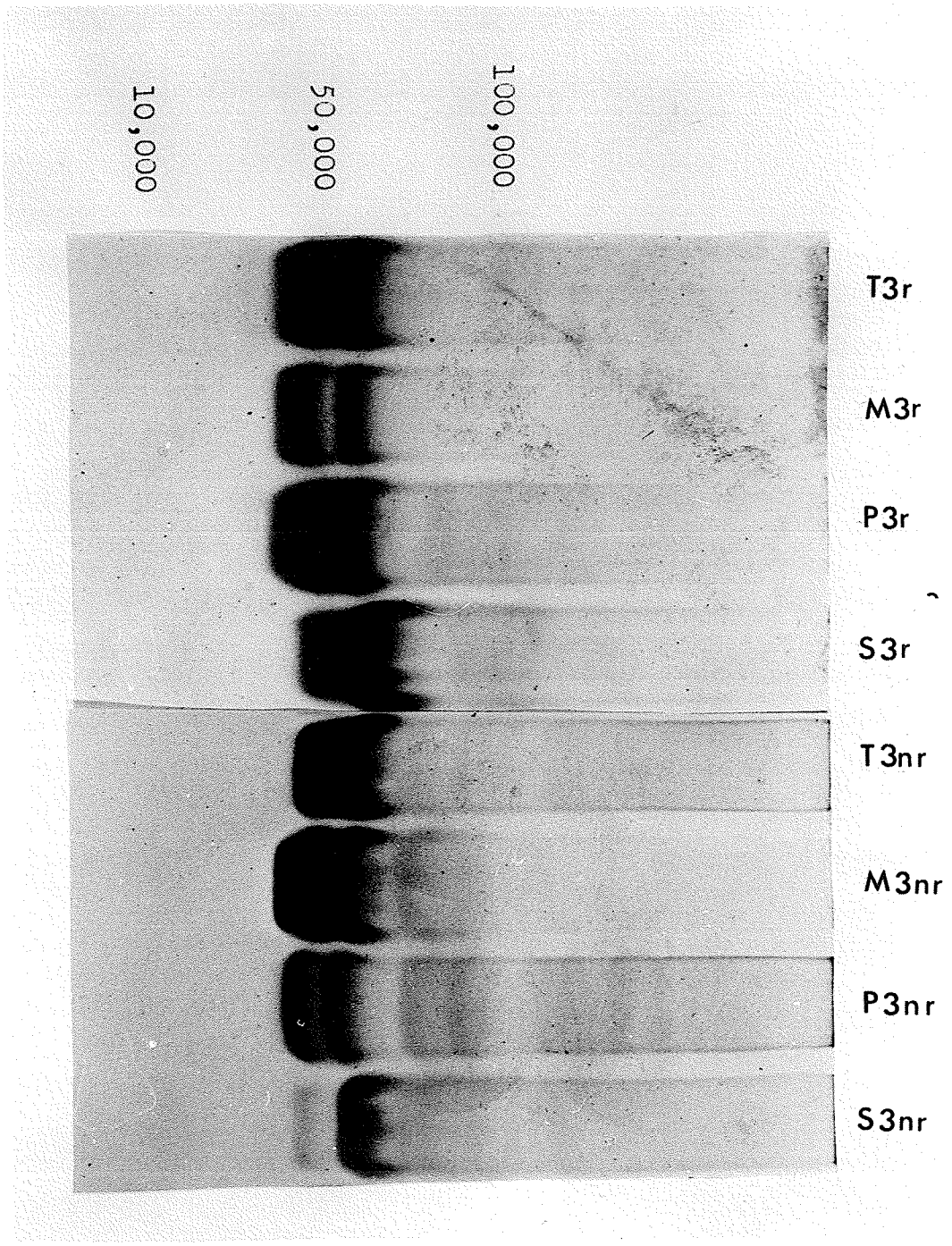
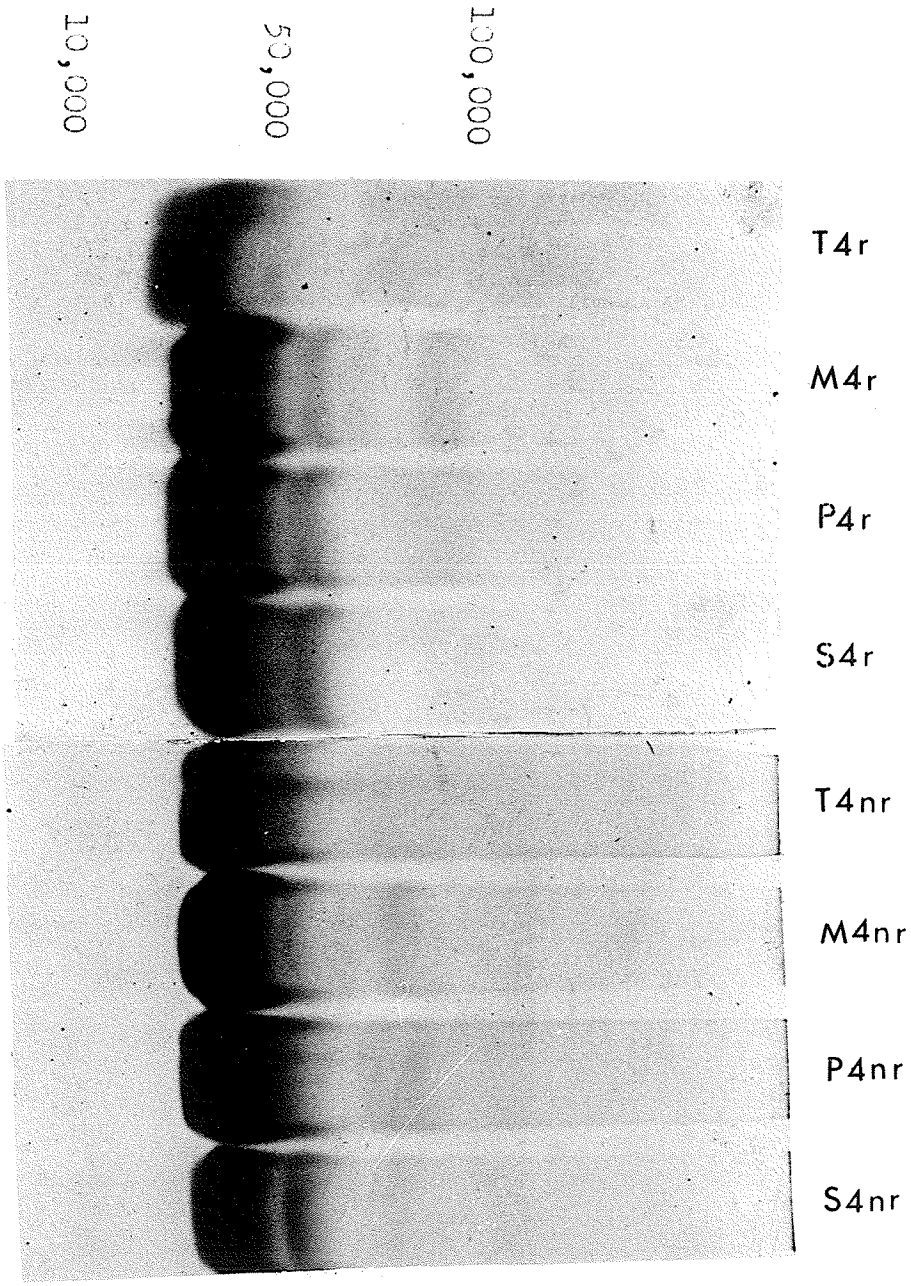

10,000
50,000
100,000
T3r
M3r
P3r
S3r
T3nr
M3nr
P3nr
S3nr

Fig. 8. SDS-PAGE patterns of reduced (r) and non reduced (nr) fraction 4 gliadins of Talbot (T), Manitou (M), Pembina (P) and Stewart 63 (S) from gel filtration.


100,000
50,000
10,000
T4r
M4r
P4r
S4r
T4nr
M4nr
P4nr
S4nr

tetraploid varieties also had minor subunits with MW's of 78,000 and 82,000 while the hexaploids had an additional minor subunit of MW 88,000.

Attempts to characterize the low molecular weight (F_5) gliadins by SDS-gel electrophoresis were unsuccessful due to streaking. However, the reduced whole gliadin of each variety had a strong band of MW 10,000 absent in the other four gliadin fractions. This band is probably the only subunit present in the fraction 5 gliadins.

General Discussion

Although only four varieties from three different wheat classes (2 HRS, 1 SWW and 1 amber durum) were studied, the similarity in their SDS-gel patterns suggests that gliadins from different classes of wheat all have polypeptide chains of similar molecular weight. Both the hexaploid (AABBDD) and tetraploid (AABB) varieties had major gliadin subunits of MW's 10,000, 36,000, 40,000 and 50,000 and minor subunits of MW's 53,000, 78,000, 82,000, and 108,000. The hexaploid varieties had additional minor subunits of MW's 88,000, 120,000 and 130,000. These additional minor subunits are probably controlled by genes on the D-genome. Electrophoretic studies of compensating nullisomic-tetrasomic stocks of Chinese Spring wheat and of Canthatch (AABBDD) - Tetracanthatch (AABB) have shown that the D-genome controls several slow moving gluten proteins (96,97).

The small number of different molecular weight wheat gliadin subunits identified in this paper and by Bietz and Wall (44) is in contrast

to the large number of gliadin proteins identified by combining gel electrophoresis and electrofocusing (102). It is generally accepted that the three genomes of wheat were derived from a common diploid progeniter (123). Each genome would therefore be expected to have genes related by common ancestry coding for gliadin polypeptide chains of similar molecular weight. However these genetically related gliadin polypeptide chains would probably have evolved different amino acid compositions. These differences in amino acid composition would lead to altered electrophoretic mobilities and isoelectric points resulting in an increased number of electrophoretically separable bands.

The wheat gliadin subunits identified by SDS-gel electrophoresis represent reduced and denatured polypeptide chains. In contrast, the structure of intact gliadin proteins are stabilized by disulfide bonding. The high molecular weight gliadin fractions (F_1, F_2) isolated by gel filtration consist of disulfide cross-linked polypeptide chains of MW's 40,000, 50,000 and 53,000 while the low molecular weight fractions consist entirely of single polypeptide chains stabilized by intra-chain disulfide bonding. At the present time it is not known if the subunits of high molecular weight gliadins are identical to the subunits in the low molecular weight fractions with the same molecular weights. It is probable that the subunits of the high molecular weight gliadins were evolved from those present in the low molecular weight fractions by amino acid mutations which increased inter-chain disulfide bonding by changing the conformation of the polypeptide chains. Ewart has presented a similar argument for the origin of disulfide cross-linking in glutenins (124).

The amino acid compositions of the wheat gliadin fractions do not show such clear differences as in rye (119). The first four wheat gliadin fractions isolated by gel filtration had similar amino acid compositions. In contrast the rye gliadin fractions were characterized by an increase in glutamic acid and proline and a decrease in lysine, arginine, aspartic acid and glycine as molecular weight increased. But more significant, in rye about 50% of the gliadins were composed of subunits of MW 110,000 whereas in wheat only minor amounts of large subunits are present. Thus in wheat the high molecular weight gliadin fractions is made up of smaller subunits with amino acid compositions similar to the lower molecular weight gliadin fractions.

Genetic Relationships in the Triticinae: Comparison Of Gliadin and Glutenin Subunits of T. Monococcum, Ae. Squarrosa, T. Aestivum and S. Cereale By SDS-Polyacrylamide Gel Electrophoresis

Abstract

The subunit molecular weights of gliadin and glutenin protein subunits from T. monococcum, Ae. squarrosa, T. aestivum and S. cereale were compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Seven gliadin subunits and nine glutenin subunits were common to T. monococcum (AA), Ae. squarrosa (DD) and T. aestivum (AABBDD). An additional five gliadin and five glutenin bands present in T. aestivum were present in either T. monococcum or Ae. squarrosa. S. cereale had a lower number of gliadin and glutenin subunits. However, three of four gliadin and eight of nine glutenin subunits present in S. cereale had molecular weights identical with subunits present in the Triticum-Aegilops group. These results have been used to consider genetic relationships in the Triticinae.

Introduction

A number of studies have utilized electrophoretic analysis of proteins and enzymes to evaluate evolutionary relationships within the Triticinae (81,82,96,125). In these techniques, separation of protein depends upon

differences in their electrophoretic mobility which is determined by their charge, size and conformation. Electrophoretic studies of gliadins and glutenins are especially difficult due to their low content of charged amino acids resulting in low mobility and hence limited resolution. An additional problem in the study of these proteins is their tolerance to amino acid substitutions. Ewart suggested that the gluten proteins of cereals are tolerant to amino acid substitutions which change the conformation or the charge on these proteins since they act mainly in a storage capacity (126). Similarly, if structure is not essential to function, the proteins should be tolerant of the effects of unequal crossing over which would cause an increase or decrease in the size of the protein. Elton and Ewart (11) and Graham (127) have shown that significant varietal variation occurs in the electrophoretic patterns of gluten proteins of T. aestivum. Boyd et al. (96) have shown that intraspecific variation in the electrophoretic patterns of the gluten proteins of T. monococcum, T. aegilopoides and Ae. squarrosa is almost as great as interspecific variations. However, these studies were not carried out on disulfide reduced proteins. It is possible some of the variation might be due to conformational isomers due to different sites of disulfide bonding.

Sodium dodecyl sulfate (SDS) binds to reduced proteins at a constant SDS-protein ratio resulting in a constant mass to charge ratio which masks native charge on the protein (128). In addition the complex appears to assume a constant helical conformation (128). Since charge and conformation are fixed, electrophoretic mobility depends only upon the size of the protein. This technique has found wide application as a rapid and simple means of

estimating molecular weights of proteins (122,129).

SDS-polyacrylamide gel electrophoretic studies have shown that there is little varietal variation in the subunit structure of gliadins or glutenins in hexaploid wheats (44, 111, 130). Gliadin consists of approximately 11 subunits ranging in molecular weight from 10,000 to 130,000 while glutenin contains approximately 15 subunits ranging in molecular weight from 11,000 to over 130,000 (44,111,130). Since two of the three genomes in hexaploid wheat are believed to be derived from Triticum monococcum (AA) and Aegilops squarrosa (DD) (131), it may be inferred that their gliadin and glutenin subunit structures would be similar to those of hexaploid wheats. In addition cytogenetic evidence indicates that the Triticum-Aegilops group and Secale cereale were derived from a common diploid progenitor (108). Accordingly these diploids would be expected to have similar subunit structures. In the present study, the gliadins and glutenins of several varieties of T. monococcum, Ae. squarrosa, S. cereale and T. aestivum have been compared by SDS-polyacrylamide gel electrophoresis to determine genetic relationships in the Triticinae.

Materials and Methods

Four accessions (varieties) of T. monococcum (2B26, 5317, 5244 and 2B7), three of Ae. squarrosa (5271,5266 and 2C48), four of S. cereale (cv. Prolific, Argentine, Explorer and Apizaco) and one of T. aestivum (cv. Manitou) used in this study were grown in the greenhouse from seed stock available in the Department of Plant Science, University of Manitoba.

Approximately 10 g of seed from each variety was ground in a coffee grinder and then defatted with chloroform-benzene (52:18^{v}/v). Gliadin and glutenin were isolated from the defatted ground grain by the modified Osborne procedure of Chen and Bushuk (109).

Proteins (10 mg/ml) were reduced by reaction with 1% 2-mercaptoethanol in pH 7.3 phosphate buffer (0.015 M) overnight at 45°C in the presence of 1% SDS. Electrophoresis was performed on a 5% polyacrylamide gel with pH 7.3 phosphate buffer (0.015 M) containing 0.01% SDS according to the method of Orth and Bushuk (111). Gels were stained in Coomassie Brilliant Blue R250 by the method of Koenig et al (112). Subunit molecular weights were determined by calibration of the gels with Manitou gliadin and glutenin whose subunit molecular weights were previously determined (111,130).

Results and Discussion

Diagrams of the SDS-polyacrylamide gel electrophoretic patterns of the gliadins and glutenins of T. monococcum, Ae. squarrosa, S. cereale and T. aestivum are shown in Figure 9. The estimated molecular weights of their subunits are shown in Table 9. Although the estimated molecular weights of the gliadin and glutenin subunits obtained in the present study are different (± 10%) than those reported in previous studies (44,111,132, 133), it should be noted that these differences are probably due to limitations in the determination of molecular weights (122) rather than representing different subunits.

Fig. 9. Diagram of SDS-PAGE patterns of reduced gliadins and glutenins of T. monococcum, Ae. squarrosa, S. cereale and T. aestivum.

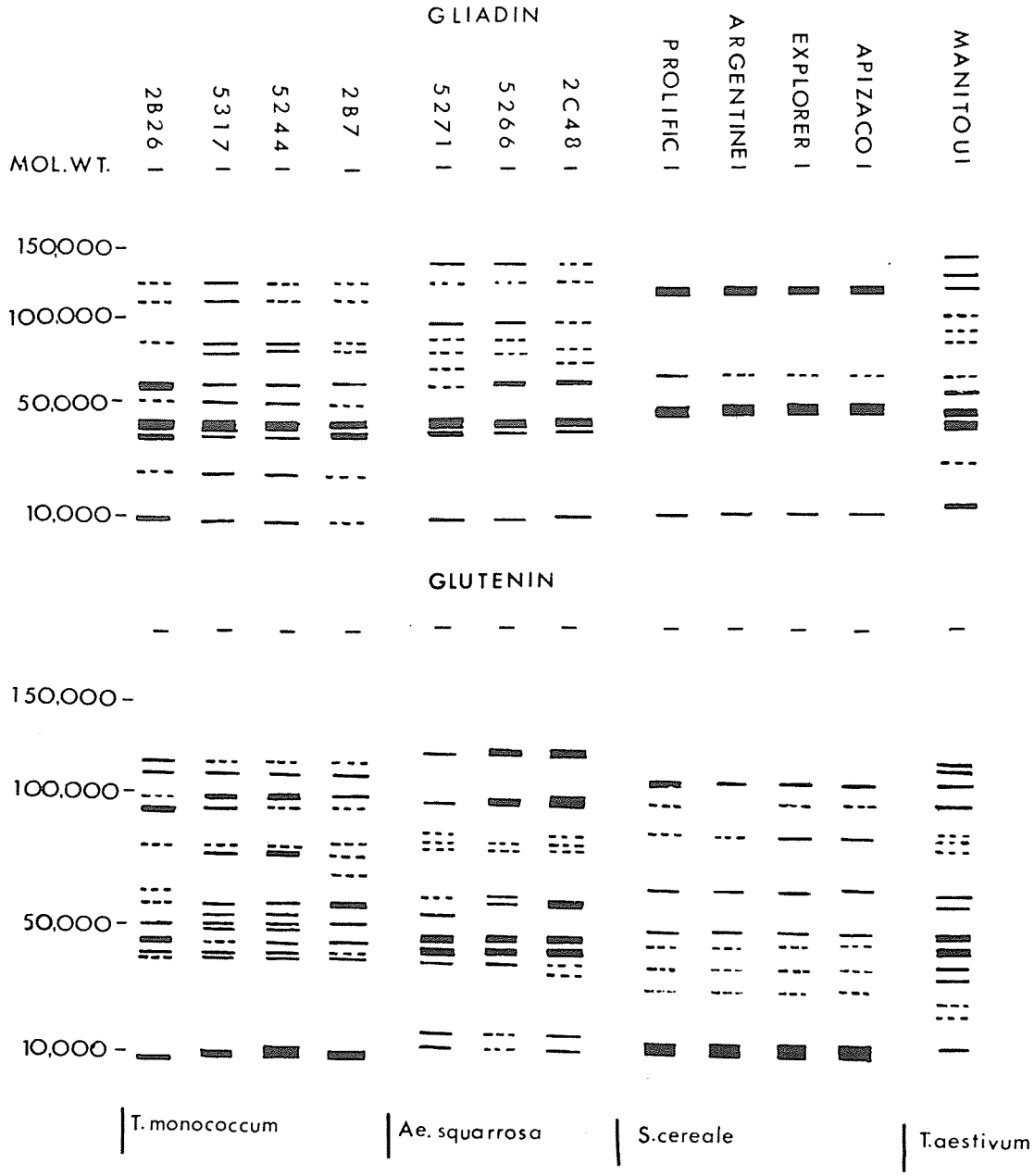

GLIADIN
MOL.WT.
2B26 I
5317 I
5244 I
2B7 I
5271 I
5266 I
2C48 I
PROLIFIC I
ARGENTINE I
EXPLORER I
APIZACO I
MANITOU I
150,000-
100,000-
50,000-
10,000-
GLUTENIN
150,000-
100,000-
50,000-
10,000-
T. monococcum
Ae. squarrosa
S.cereale
T.aestivum

Table 9. Calculated molecular weights of gliadin and glutenin subunits in T. monococcum, (T. mono.), Ae. squarrosa (Ae. sq.), T. aestivum (T. aest.) and S. cereale (S. cer.)

Subunit Molecular Weight	Gliadin*				Glutenin*			
	T. mono.	Ae. sq.	T. aest.	S. cer.	T. Mono	Ae. sq.	T. aest.	S. cer.
130,000		M	M			S	M	
120,000	M	F	M		F		M	
108, "	M		M	S	M		M	M
100, "					M			
95, "					M	S	M	
88, "		M	F			F	F	F
82, "	M	F	F		F	F	F	M
78, "	M	F	F		M	F	F	
70, "					F			
64, "		F			F			M
58, "					M	M	M	
56, "	M	M	F	F	M	M	M	
50, "	M		M		M			
46, "					M			M
42, "				S				
40, "	S	S	S		M	S	S	F
36, "	S	S	S		M	S	S	
34, "					M	M	M	F
30, "						F	M	
24, "	F		F					F
21, "							F	
19, "							F	
14, "						M		
10, "	M	M	M	M	S	M	M	S

* Band intensities are marked S (strong), M (medium), F (faint)

Intervarietal variation in the gliadin and glutenin subunit patterns of T. monococcum, Ae. squarrosa and S. cereale was small. The gliadin and glutenin subunit structures of T. monococcum and Ae. squarrosa were also similar. Seven gliadin bands (MW's 120,000, 82,000, 78,000, 56,000, 40,000, 36,000 and 10,000) and nine glutenin bands (MW's 95,000, 82,000, 78,000, 58,000, 56,000, 40,000, 36,000, and 10,000) were common to both species. Since the molecular weight of each subunit (representing a polypeptide chain) is proportional to the number of amino acids present and thus proportional also to the length of the chromosomal gene coding for that subunit, the number of similar subunits found in different species should give an indication of their genetic relatedness. Thus the close similarity between the gliadin and glutenin subunit structures of T. monococcum and Ae. squarrosa suggests that these species are closely related and that the majority of their polypeptide chains are coded for by homoeologous genes, thus supporting cytogenetic evidence (131) that the diploid progenitor of the A (T. monococcum) and D (Ae. squarrosa) genomes in hexaploid wheats were derived from a common diploid progenitor.

The subunit patterns of the gliadins and glutenins of T. aestivum (cv. Manitou) were very similar to those of T. monococcum and Ae. squarrosa. The seven gliadin and nine glutenin bands present in both T. monococcum and Ae. squarrosa were also present in T. aestivum. Thus in T. aestivum each of these bands probably represents several polypeptide chains of similar molecular weight controlled by genes on both the A and D genomes. In addition five gliadin and five glutenin bands in T. aestivum were unique to

either T. monococcum or Ae. squarrosa. Thus gliadin subunits in T. aestivum of MW's 108,000, 50,000 and 24,000 and glutenin subunits of MW's 120,000 and 108,000 present also in T. monococcum are probably controlled by genes on the A genome while gliadin subunits of MW's 130,000 and 88,000 and glutenin subunits of MW's 130,000, 88,000 and 30,000 are probably controlled by genes on the D-genome. Recently Orth and Bushuk (132,133) found that four glutenin subunits are controlled by genes on the D-genome. Unfortunately the diploid from which the B-genome was derived is not known (134) and hence could not be included in the present study.

Cytogenetic evidence indicates that S. cereale is derived from the same diploid progenitor as are the donors of the three genomes of wheat (108). Recent studies have shown that the three genomes of hexaploid wheat each have two chromosomes associated with the genetic control of gliadin proteins (97) while in S. cereale only one chromosome is involved (99). Shepherd and Jennings have postulated that the ancient diploid which was the common progenitor of S. cereale and the diploid donors of the A, B and D genomes in hexaploid wheat had only one chromosome associated with the control of gliadin synthesis and that a descendent from which the donors of the wheat genomes evolved acquired two-chromosome control by gene duplication and translocation (99).

In the present study S. cereale was found to have fewer gliadin and glutenin subunits than did T. monococcum and Ae. squarrosa. However three of the four gliadin subunits and eight of the nine glutenin subunits present in S. cereale had identical molecular weights with subunits present in T. monococcum and/or Ae. squarrosa. The similarity of the

subunit structure of S. cereale and of the two diploid donors of the A and D genome suggests that these species are related and thus supports cytogenetic evidence (108) that these diploids were derived from a common ancient diploid progenitor. Since the number of loci controlling protein phenotype is a reliable index of evolutionary divergence (135, 136), the difference in the number of gliadin and glutenin subunits in S. cereale compared to T. monococcum and Ae. squarrosa suggest that these species have evolved by different routes. Shepherd and Jennings hypothesis of gene duplication and translocation in the most plausible explanation of the present results. This process could lead to additional gliadin and glutenin subunits in the Triticum-Aegilops group not present in S. cereale if the duplication and/or translocation of genes coding for these proteins involved uneven crossing over.

The Effects of Gliadin, Albumin and Gliadin Fractions Isolated by Gel Filtration on the Mixing Properties of a Synthetic Dough System

Abstract

A synthetic dough system consisting of gliadin (cv. Manitou), glutenin and starch gave a strong mixing curve. When gliadin was replaced by high molecular weight gliadin (MW 100,000) isolated by gel filtration, a slightly stronger mixing curve was obtained. In contrast, replacement of gliadin with gliadin fractions of lower molecular weights (MW's 44,000 and 27,000) gave much weaker mixing curves. The weakest mixing curve was obtained when albumin (cv. Manitou) was substituted for gliadin.

Introduction

Smith and Mullen (8,137) have shown that the differences in the mixing characteristics of short- and long-mixing flours appear to be determined by the protein-starch residue fraction and by both the quantity and molecular weight distribution of the water soluble gliadins. Murthy and Dahle (138), using a model dough system containing gliadin, glutenin and wheat starch, found that pretreatment of the gliadin with N-ethylmaleimide or dithiothreitol weakened the mixing curve obtained on the farinograph. However no studies are available on the effects of individual

gliadin components on mixing properties. Recently Preston and Woodbury (119,130) have shown that the alcohol soluble (gliadins) proteins of wheat and rye can be separated by gel filtration into four distinct fractions varying in molecular weight. In the present study the effects of these fractions on the mixing properties of a model dough system have been studied on the electronic recording dough mixer, which has been described previously (139,140).

Materials and Methods

Isolation of Gliadin, Glutenin and Albumin. Gliadin and albumin were isolated from Manitou (hard red spring) flour by the modified Osborne procedure of Chen and Bushuk (109) and then washed with chloroform-benzene (52:18^{v}/v) to remove lipids. Glutenin was isolated from commercial vital wheat gluten (hard red spring) by the method of Murthy and Dahle (138). Protein contents of the gliadin, glutenin and albumin were 84.0, 79.2 and 65.0% respectively (N x 5.7, Kjeldahl method). Wheat starch was obtained from Industrial Grain Products, Thunder Bay, Ontario. The vital gluten was tested for denaturation by AACC method 28-20 and found to be undenatured.

Isolation of Gliadin Fractions. Manitou gliadin isolated as described above was fractionated on a Sephadex G-100 column as previously described (119,130). Sufficient quantities of three of the four gliadin fractions were isolated for further study. These fractions had average molecular weights of 100,000, 44,000 and 27,000. After washing each gliadin fraction with chloroform-benzene to remove lipids and freeze-

drying, the fractions had protein contents of 92.2, 84.8 and 81.8% respectively (N x 5.7, micro Kjeldahl).

Mixing Studies. A synthetic dough system similar to that of Murphy and Dahle (138), consisting of 0.77 g gluten, 0.27 g defatted Manitou Osborne gliadin, 2.00 g wheat starch and 2.1 ml. water was mixed in the electronic recording dough mixer (5. g bowl, 92 r.p.m.) as described by Voisey and Miller (141). Glutenin, gliadin and wheat starch were weighed separately into a 10 ml vial and mixed, then added to the mixing bowl. The protein content of this synthetic flour system was 24.5%. The water was added and the dough mixed for 10 minutes. Mixing curves of this synthetic dough system were repeated a number of times to check reproducibility. The consistency of the dough (g-cm) varied less than 5% for five trials.

The effects of the alcohol soluble (gliadin) fractions isolated by gel filtration and of Osborne albumin in the mixing properties of the synthetic dough system were studied by substituting each gliadin fraction and albumin on an equal protein basis for the total alcohol-soluble fraction while keeping the amounts of glutenin, wheat starch and water constant. Table 10 summarizes the composition of each dough system used in the present study.

Results

In the present study the proportions of glutenin, starch and water in the synthetic dough system were kept constant. Thus any differences

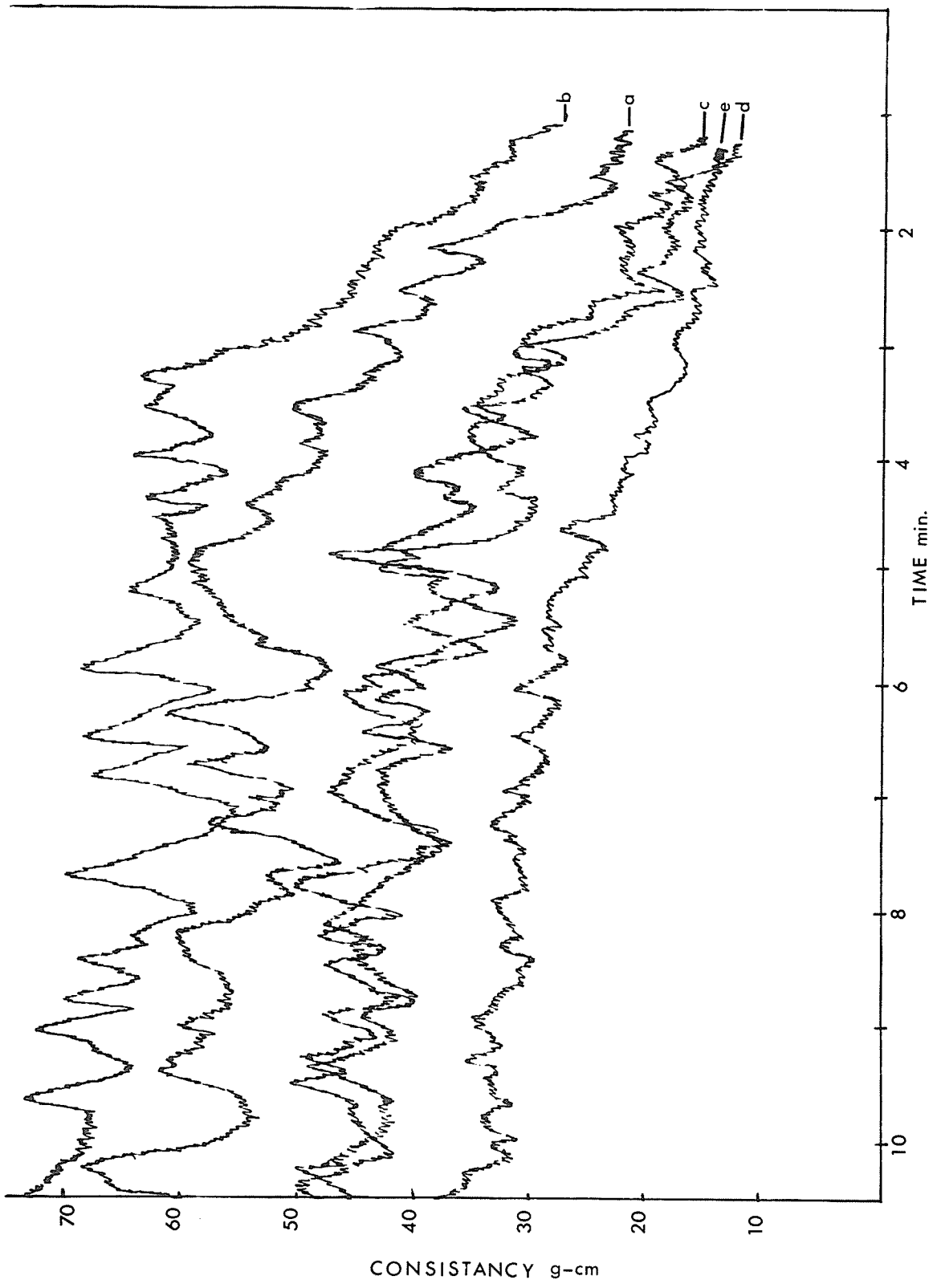
Table 10. Composition of Synthetic dough systems[a]

Dough System	Glutenin(g)	Starch(g)	Water(ml)	Variable Component	Weight(g)
A	0.77	2.00	2.1	Osborne Gliadin	0.27
B	0.77	2.00	2.1	M_1 Gliadin[b]	0.25
C	0.77	2.00	2.1	M_3 Gliadin[b]	0.27
D	0.77	2.00	2.1	M_4 Gliadin[b]	0.28
E	0.77	2.00	2.1	Osborne Albumin	0.35

[a] M_1, M_3, M_4 refer to Manitou Osborne gliadin fractions isolated by gel filtration with MW's of 100,000 and of 44,000 and 27,000 respectively

[b] each synthetic flour system contains 24.5% protein

Fig. 10. Tracings of the mixing curves of a synthetic dough system containing glutenin, starch, water and a) Osborne gliadin b) gliadin (MW approximately 100,000) c) gliadin (MW = 44,000) d) gliadin (MW = 27,000) e) Osborne albumin.


b
a
c
e
d
70
60
50
40
30
20
10
CONSISTANCY g-cm
2
4
6
8
10
TIME min.

in the mixing curves could be directly attributed to the variable component. Tracings of the mixing curves of the synthetic dough systems studied on the electronic recording dough mixer are shown in Figure 10.

The synthetic dough systems containing the total alcohol soluble Manitou flour proteins (gliadins) and the high molecular weight gliadin fraction (MW 100,000) obtained by gel filtration gave strong mixing curves. The mixing curve of the dough system containing the high molecular weight fraction was slightly stronger. The two lower molecular weight fractions (MW's 44,000 and 27,000) were less effective in strengthening the synthetic doughs. The weakest mixing curve was obtained when the Manitou albumin (water-soluble) fraction was used instead of the alcohol soluble fraction.

General Discussion

Previous studies have indicated that the molecular weight distribution of gluten proteins are important in determining the rheological properties of dough. The results of Smith and Müllen (8) indicated that the differences in the molecular weight distribution of the gliadin components of a short- and long-mixing flour were partly responsible for their different rheological properties. More recently Lee and MacRitchie (142) found that gluten proteins of high molecular weight added to a base flour strengthened the dough. In the present study, the synthetic dough system containing the high molecular weight Osborne gliadin (MW greater

than 100,000) gave much stronger mixing curves than the synthetic doughs containing the lower molecular weight fractions (MW's 44,000 and 27,000). These results indicate that the molecular weight distribution of gliadin components in wheat flour could contribute to differences in the rheological properties of different quality wheats.

When gliadin was replaced by Osborne albumins very weak mixing curves were obtained. Apparently the absence of gliadin in doughs results in a lack of extensibility and cohesiveness (143) which must be present to give dough its normal rheological properties.

The study reported in this paper involved a simplified dough system containing starch, glutenin and gliadin. Thus it must be kept in mind that a natural dough system is much more complex. However the advantage of working with the synthetic system is that the concentrations of natural components can be varied to accentuate their effects by either deletion or addition.

Characterization of the Residue Proteins in a Hard Red Spring Wheat

Abstract

Approximately 80% of the residue proteins of a hard red spring wheat (cv. Manitou) were solubilized in 0.1N acetic acid following reduction and alkylation of disulfide bonds. Sequential extraction of the solubilized proteins with 0.5M sodium chloride, 70% ethanol and 0.1N acetic acid showed that the bulk of these proteins were soluble in ethanol (47%) and acetic acid (35%). Both the alcohol and acid soluble fractions had amino acid compositions, electrophoretic patterns and subunit structures similar to reduced and alkylated glutenin. The alcohol soluble subunits had MW's 130,000, 120,000, 108,000, 95,000, 65,000 60,000 and 50,000 while the acid soluble subunits had MW's 130,000, 120,000, 108,000, 95,000, 68,000, 60,000, 56,000, 40,000 and 36,000.

Introduction

Following extraction of wheat flour by the modified Osborne procedure of Chen and Bushuk (109) or by exhaustive extraction with dilute lactic or acetic acid (63,65), a considerable portion of the protein remains insoluble. This "residue" or "gel" protein accounts for approximately 15 to 35% of the total flour protein (9,67). Several studies have shown that these proteins are related to the dough-mixing properties (64,144) and

loaf volume potential (9) of flour.

Inamine et al (66) solubilized approximately 85% of the insoluble protein remaining after exhaustive extraction of flour with dilute acetic acid with 0.1M aqueous dimethylaminoethanol. Chromatography of the solubilized protein on agarose columns yielded two high molecular weight fractions, both of which had electrophoretic patterns similar to glutenin after reduction and alkylation. In contrast, Cluskey and Dimler (65) found that the acetic acid insoluble flour proteins had electrophoretic patterns and amino acid compositions more closely resembling the water soluble flour proteins than glutenin. Mecham et al (67) found that much of the residue flour protein could be solubilized with 0.05 mM mercuric chloride in 0.01M acetic acid. In seven flours, 12 to 28% of the total flour protein was solubilized. Cole et al (145) analyzed the mercuric chloride solubilized residue proteins by ultra centrifugation and showed the bulk of these proteins had molecular weights of 40,000 to 44,000. Recently Cole et al (68) were able to separate mercuric chloride solubilized residue proteins on agarose columns into gel-filtered and salt eluted fractions. Both fractions had amino acid compositions similar to glutenin. Electrophoresis in the presence of sodium dodecyl sulfate (SDS) showed that the salt eluted fraction had major subunits with molecular weights of 46,000, 70,000 and 88,000.

In the present paper the characterization of the residue proteins in a hard red spring wheat (cv. Manitou) was undertaken. In contrast to previous experiments, residue proteins were first solubilized by reduc-

tion and alkylation and the protein fractionated on the basis of its solubility. A study of the amino acid composition, electrophoretic properties and subunit structure of each solubility fraction was undertaken.

Materials and Methods

Isolation and Fractionation of Residue Proteins. The flour used was straight grade, milled on a Buhler experimental mill from the hard red spring wheat variety, Manitou. The flour was fractionated by the modified Osborne procedure of Chen and Bushuk (109) and the resulting 0.1M acetic acid insoluble residue fraction was isolated. This fraction was resuspended in excess 0.1N acetic acid at 4°C and stirred overnight then centrifuged. The resulting precipitate was lyophilized and 8.0 g of the dried residue was reduced with 2-mercaptoethanol and alkylated with acrylonitrile in 6M urea by the method of Crow and Rothfus (52). Following reduction and alkylation the solution was centrifuged and both the precipitate and supernatant were dialyzed exhaustively against 0.1N acetic acid and lyophilized. A dry sample from the soluble portion (500 mg) was then sequentially extracted with two 10 ml portions of 0.5 M sodium chloride, 10 ml distilled water, two 10 ml portions of 70% ethanol and two 10 ml portions of 0.1N acetic acid using the procedure of Chen and Bushuk (109). The salt and water soluble extracts were combined and dialyzed against distilled water. Ethanol was removed from the 70% ethanol soluble solution by evaporation under vacuum. Each fraction was then frozen and lyophilized. Osborne albumin, gliadin and glutenin

isolated from Manitou flour by the procedure of Chen and Bushuk (109) were reduced and alkylated as described above. Protein (N x 5.7) was determined by the macro- or micro-Kjeldahl procedure. Protein yields from the various isolation procedures are shown in Table 11.

Amino Acid Analysis. Amino acid analysis was performed on a Beckman model 121 amino acid analyzer by the method of Spackmann et al (113). Hydrolysates were prepared for analysis by the method of Tkachuk (146). Dried hydrolysates were dissolved in pH 2.2 citrate buffer (0.1M), centrifuged to remove humin, then applied to the ion-exchange column of the analyzer. Tryptophan and cystine values were not determined.

Polyacrylamide Gel Electrophoresis and SDS-Polyacrylamide Gel Electrophoresis. Electrophoresis was carried out in a 7.5% polyacrylamide gel according to the procedure of Chen and Bushuk (106). SDS-polyacrylamide gel electrophoresis was carried out as previously described (119).

Results and Discussion

Solubility Fractionation of Reduced and Alkylated Residue Protein. Fractionation of the Manitou flour proteins by the modified Osborne procedure of Chen and Bushuk (109) yielded an insoluble fraction which accounted for 30% of the total flour protein. In order to remove entrapped soluble proteins, this fraction was further extracted overnight in 0.1N acetic acid. Approximately 14% of the insoluble protein was solubilized by this additional extraction. The remaining insoluble fraction (protein content = 4.5%) was subjected to further examination.

Table 11. Qualitative distribution of protein (N x 5.7) from Osborne and reduced and alkylated residue fractionations.

Fraction	% Total Flour Protein	% Total Protein of Fraction
Total Flour	100	
Water soluble		12
Sodium Chloride soluble		5
Ethanol soluble		38
Acetic Acid soluble		15
Insoluble		30
Osborne Insoluble	30	
Acid soluble		17
Acid insoluble		83
Reduced and Alkylated Residue[1]	20	
Water soluble		2
Salt soluble		0
Alcohol soluble		47
Acid soluble		35
Insoluble		12

[1] Refers to reduced and alkylated residue soluble in 0.1N acetic acid following dialysis (see text).

Reduction of disulfide bonds with 2-mercaptoethanol and alkylation with acrylonitrile in 6M urea solution solubilized 89% of the residue protein. Approximately 10% of the solubilized protein precipitated following exhaustive dialysis against 0.1N acetic acid while 5% of the protein was lost during dialysis. The material soluble in acetic acid was lyophilized and fractionated by sequential extraction with 0.5M sodium chloride, 70% ethanol and 0.1N acetic acid. The bulk of the reduced and alkylated residue protein was found in the ethanol and acetic acid soluble fractions which accounted for 47 and 35% of the total protein respectively. Approximately 2% of the protein was soluble in sodium chloride and remained in solution after dialysis with distilled water while 12% of the protein was insoluble in all of the above solutions. This insoluble fraction was not further studied.

Amino Acid Composition. The amino acid composition of the Osborne solubility fractions of the soluble reduced and alkylated residue proteins are shown in Table 12. Also included in the table are the amino acid compositions of reduced and alkylated Manitou albumin, gliadin and glutenin. The ethanol soluble fraction had an amino acid composition more similar to wheat glutenin than gliadin. This is especially evident in the case of glycine. Previous studies by Ewart have shown that wheat gliadin has a much lower content of glycine than has glutenin (47), as is evident in Table 12. The acetic acid soluble fraction had less glutamic acid and proline than the ethanol soluble fraction with an amino acid composition intermediate to Osborne glutenin and albumin.

The salt soluble fraction had an amino acid composition similar to the 0.1N

TABLE 12. Amino acid composition of solubility fractions of reduced and alkylated residue proteins and reduced and alkylated albumin, gliadin and glutenin (mole %)*

Amino Acid	Reduced and Alkylated Residue Fraction						
	Residue[a]	0.5M sodium chloride	70% ethanol	0.1N acetic acid	Albumin	Gliadin	Glutenin
Lysine	2	2	1	2	3	1	1
Histidine	2	2	2	2	2	1	
Ammonia	36	38	37	32	32	41	39
Arginine	2	3	2	3	4	1	2
Aspartic acid	3	4	2	4	7	3	2
Threonine	3	3	3	3	4	2	2
Serine	7	6	7	6	6	5	5
Glutamic acid	36	34	38	33	24	40	40
Proline	12	13	13	12	11	18	16
Glycine	8	10	8	8	7	3	7
Alanine	4	4	3	4	7	3	3
Valine	4	4	4	5	6	4	3
Methionine	1	1	1	2	2	1	1
Isoleucine	3	2	3	3	3	3	3
Leucine	7	6	7	7	8	7	6
Tyrosine	3	3	3	3	3	3	2
Phenylalanine	3	3	3	3	3	3	5

*Cystine and tryptophan not determined

[a]Residue refers to protein soluble in acetic acid after reduction and alkylation

Fig. 11. PAGE patterns of reduced and alkylated acid-soluble (a), ethanol-soluble (c) and water-soluble (e) residue proteins and reduced and alkylated Osborne glutenin (b), gliadin (d), albumin (f) and globulin (g) from Manitou flour.

a b c d e f g

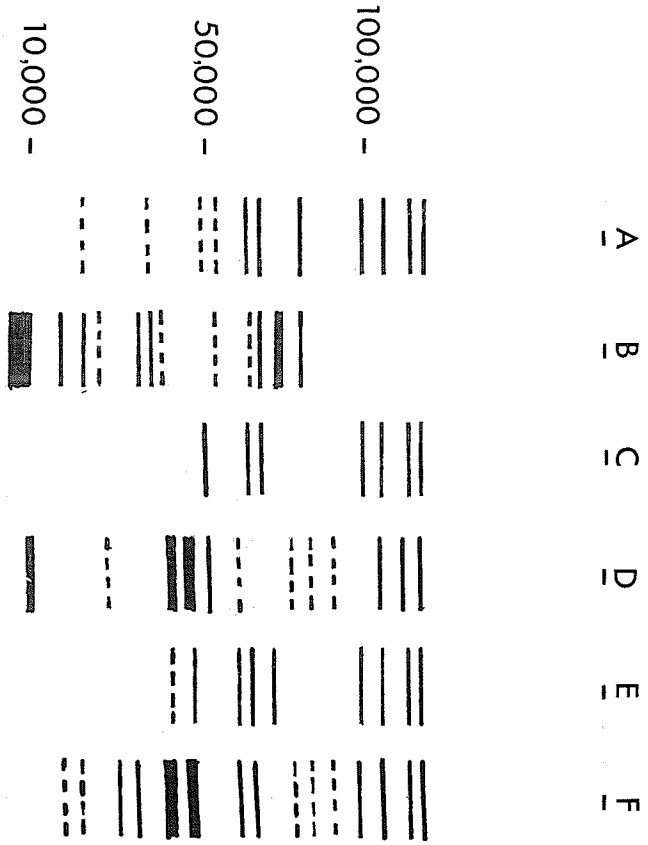
acetic acid soluble fraction. In order to determine if substantial amounts of urea were bound to the reduced and alkylated residue fraction, ammonia contents were included (Table 12). The ratio of glutamic acid plus aspartic acid to ammonia in the residue fractions is similar to those of Osborne gliadin and glutenin, indicating that very little urea is present in the residue fractions.

From the above data it is evident that the residue proteins solubilized by reduction and alkylation in Manitou flour resemble both albumin and glutenin in their amino acid compositions. Similar results were recently reported by Cole et al (68) who found that the acetic acid-insoluble proteins of a Kansas hard red winter wheat had an amino acid composition similar to glutenin.

Polyacrylamide Gel Electrophoresis. Further characterization of the solubilized residue protein fractions was carried out by polyacrylamide gel electrophoresis. Figure 11 shows the electrophoretic patterns of these fractions. Also included in Figure 11 are patterns for reduced and alkylated Manitou albumin, gliadin and glutenin. The ethanal soluble and acetic acid soluble fractions had electrophoretic patterns closely resembling reduced and alkylated glutenin. These fractions also contained some faint fast moving bands. Differences in the staining intensities of the two fractions and of glutenin indicated that the quantitative distribution of components was different. The water soluble fraction had only four bands of low mobility. However these bands corresponded with bands present in reduced and alkylated glutenin.

SDS-Polyacrylamide Gel Electrophoresis. Patterns of the SDS-polyacrylamide gel electrophoretic patterns of the solubility fractions of reduced and alkylated soluble residue proteins and of reduced and alkylated Manitou Osborne albumin, gliadin and glutenin are shown in Figure 12. The ethanol soluble fraction had subunits with MW's 130,000, 120,000, 108,000, 95,000, 65,000, 60,000, and 50,000 while the acid soluble fraction had subunits with MW's 130,000, 120,000, 108,000, 95,-000, 68,000, 60,000, 56,000, 40,000 and 36,000. Both fractions had sub-unit structures more closely related to reduced and alkylated glutenin than to reduced and alkylated gliadin or albumins. However several sub-units were present (in the alcohol (MW = 65,000) and acid (MW = 68,000) soluble fractions) which were not detectable in reduced and alkylated Osborne gliadin, glutenin or albumin. In addition minor subunits of MW's 78,000, 82,000 and 88,000 and three subunits with MW's less than 36,000 which were present in Osborne glutenin were absent in the soluble residue fractions. Previous studies by Tanaka and Bushuk (147) and Orth and Bushuk (unpublished data) have shown that the proteins insoluble in AUC (0.1N acetic acid, 3M urea, 0.01M cetyltrimethyl ammonium bromide) had subunits similar to glutenin. However it is difficult to compare their results to those presented in the present paper due to the difference in extraction procedures. The water soluble reduced and alkylated residue fraction had subunits present in both Osborne glutenin and albumin.

Fig. 12. Diagram of SDS-PAGE patterns obtained from reduced and alkylated water-soluble (A), alcohol-soluble (C) and acid-soluble (E) residue proteins and reduced and alkylated Osborne albumin (B), gliadin (D) and glutenin (F).


A
B
C
D
E
F
100,000 –
50,000 –
10,000 –

General Discussion

Approximately 87% of the insoluble residue proteins of Manitou flour could be solubilized by reduction and alkylation of disulfide bonds. Of the solubilized protein, 47% was soluble in 70% ethanol and 35% was soluble in 0.1N acetic acid. Both fractions had amino acid compositions, electrophoretic patterns and subunit structures resembling reduced and alkylated glutenin. However the presence of unique subunits in the alcohol (MW = 65,000) and acid soluble fractions (MW = 68,000) of the residue proteins which were not present in reduced and alkylated glutenin, gliadin or albumin suggests that these polypeptide chains might be important in the properties of the residue proteins. The insolubility of the residue proteins and their general similarity to glutenin suggests that these proteins are very high molecular weight glutenins. It is suggested that the unique subunits present in the residue fraction may be capable of producing a larger number of inter-disulfide bonds than the glutenin subunits and that these unique polypeptide chains therefore cause a higher degree of polymerization, thus increasing molecular weight and lowering solubility.

A Hypothesis for the Natural Selection of High Levels of Glutamine and Proline in Cereal Endosperm Proteins

Abstract

The endosperm flour proteins of four varieties of wheat and one variety of rye were separated into five solubility fractions by sequential extraction with 0.5M sodium chloride, 70% ethanol and 0.1M acetic acid. Corresponding solubility fractions from each variety had similar amino acid compositions. Four of the five solubility fractions, which accounted for over 90% of the total endosperm proteins, had high contents of glutamine and proline. Glutamine and proline accounted for 35 mole % of the total amino acids in the water soluble albumins and the insoluble residue proteins and over 50 mole % in the alcohol and acid soluble proteins. A hypothesis has been proposed to account for the high incorporation of these amino acids into cereal endosperm proteins by natural selection on the basis of their ability to provide nutritional elements to the embryonic plant following germination.

Introduction

The main biological function of the endosperm of cereal grain is to serve as a reservoir of protein, carbohydrate and lipid, which upon germination, is broken down and utilized by the embryonic plant. A number of

studies have shown that seed protein content is positively correlated with early seedling vigor and in some cases with yield (148,152). Recently Lowe and Ries (152) have shown a high positive correlation ($r = 0.92^{**}$) in wheat between seed protein content and seedling dry weight after three weeks growth. Seedlings grown in the dark from high protein seeds were also able to maintain higher rates of respiration than low protein seeds. The factor(s) responsible for the greater growth and respiration of high protein seeds were found in the endosperm.

The endosperm proteins of cereal grains consist mainly of prolamines and/or glutelins (2,153). These proteins are generally characterized by high levels of glutamine and proline. Indeed, these two amino acids account for over 50% of the total protein. Ewart (126) has suggested that natural selection in cereal endosperm proteins has increased glutamine and/or asparagine levels to increase nitrogen content and to minimize energy demands in the synthesis of constituent amino acids. However no explanation for the natural selection of high proline levels in these proteins has been offered.

In the present paper the amino acid composition of the protein solubility classes from four varieties of wheat and one of rye have been compared. A hypothesis has been proposed to account for the amino acid composition of these proteins with respect to their ability to act as effective nutrients to the embryonic plant following germination.

Materials and Methods

Preparation of Samples. Four varieties representing three classes of wheat and one variety of rye were obtained from stocks available in the Department of Plant Science, University of Manitoba. The wheat varieties included were Manitou (hard red spring), Pembina (hard red spring), Talbot (soft white winter) and Stewart 63 (amber durum). The spring rye variety was Prolific.

Flour, which consists almost entirely of endosperm tissue, was obtained by milling on a Buhler experimental mill. The proteins of each flour were separated into solubility classes by the modified Osborne procedure of Chen and Bushuk (109). The five solubility classes obtained were albumins (water soluble), globulins (salt soluble), prolamines (70% ethanol soluble), soluble glutenins (0.1M acetic acid soluble) and insoluble glutenins. The protein content (N x 5.7) of each protein fraction was determined by the micro-Kjeldahl procedure.

Amino acid analyses were performed on a Beckman model 121 amino acid analyzer by the method of Spackmann et al (113). Protein samples were hydrolysed by the method of Tkachuk (146). The dried hydrolysates were dissolved in pH 2.2 citrate buffer (0.1M), centrifuged to remove insoluble humin, then applied to the ion-exchange column of the analyzer. Tryptophan and cystine contents were not determined.

Results

Solubility Distribution of Flour Endosperm Proteins. The solubility distribution of the flour proteins of the four wheat and one rye variety are shown in Table 13. Total protein recoveries varied from 90 to 98%. In order to compare relative distributions between varieties, values were normalized.

The wheat flours all contained low amounts of albumin and globulin. The prolamine fraction accounted for between 30 to 40% of the total protein while the soluble and insoluble glutenins (glutelins) accounted for 45 to 55%. Similar results were obtained by Orth and Bushuk (9) in a study of the protein solubility distribution of twenty-six wheat varieties of diverse baking quality. The rye variety, Prolific, contained significantly less gliadin and more albumin than any of the wheat varieties.

Amino Acid Composition of Solubility Classes. The amino acid composition of the five protein solubility classes of the wheat and rye flours are shown in Tables (14-18). The ammonia content of each fraction indicates that most of the glutamic acid present was in the form of glutamine. Corresponding solubility classes from the wheat and rye flours had similar amino acid compositions.

In contrast to the globulins, the other four solubility classes, which account for over 90% of the total flour proteins, had high contents of glutamic acid (glutamine) and proline. Glutamic acid and proline accounted for over 35 mole % of the total amino acid residues in the

TABLE 13. Solubility Distribution of Flour Proteins in Wheat and Rye

Fraction	Manitou	Pembina	Talbot	Stewart 63	Prolific
Total Flour					
protein content, %	13.9	14.2	11.5	11.1	10.7
fraction total protein, %	100	100	100	100	100
Albumins					
fraction total protein, %	11.9	12.0	14.8	5.4	29.8
Globulins					
fraction total protein, %	5.3	2.8	3.8	2.8	8.8
Gliadins					
fraction total protein, %	38.1	31.2	34.8	40.0	17.2
Glutenins					
fraction total protein, %	15.1	13.1	15.0	15.3	13.8
Residue					
fraction total protein, %	29.7	40.9	31.6	32.2	30.6
Nitrogen Recovery, %	91.7	93.2	90.0	98.0	92.8

TABLE 14. Amino Acid Composition of Albumins (mole %)*

Amino Acid	Talbot	Manitou	Pembina	Stewart 63	Prolific rye
Lysine	3.4	2.9	2.8	3.0	2.1
Histidine	2.1	1.8	2.0	1.7	1.5
Ammonia	21.3	22.5	21.5	21.3	25.7
Arginine	4.7	4.2	4.0	3.8	2.6
Aspartic acid	6.8	6.7	6.1	6.3	4.1
Threonine	4.1	4.0	3.9	3.9	3.2
Serine	5.7	5.8	5.8	5.9	5.1
Glutamic acid	21.5	23.3	24.0	23.3	27.7
Proline	11.1	11.3	11.1	11.4	16.6
Glycine	7.3	7.0	6.4	6.6	4.3
Alanine	7.5	7.1	6.6	6.8	4.5
Valine	6.1	6.2	6.0	6.1	10.5
Methionine	2.0	1.4	1.3	1.9	1.3
Isoleucine	3.4	3.4	3.5	3.5	3.5
Leucine	7.9	7.7	7.4	7.6	6.6
Tyrosine	3.0	2.7	2.6	2.9	1.6
Phenylalanine	3.5	3.4	3 4	3.4	4.6

*Cystine and tryptophan not determined

TABLE 15. Amino Acid Composition of Globulins (mole %)*

Amino Acid	Talbot	Manitou	Pembina	Stewart 63	Prolific rye
Lysine	6.4	5.7	6.0	5.9	4.3
Histidine	2.3	2.8	2.3	2.5	2.1
Ammonia	12.9	18.9	14.0	18.7	12.7
Arginine	6.5	6.5	6.1	7.6	4.8
Aspartic acid	9.9	8.4	9.9	8.6	7.5
Threonine	4.7	4.7	4.6	4.2	4.0
Serine	6.1	6.3	6.2	6.4	5.5
Glutamic acid	12.9	16.2	13.6	16.1	16.4
Proline	5.3	5.8	5.4	5.0	6.5
Glycine	9.3	9.1	9.1	9.8	8.1
Alanine	9.1	8.7	8.9	9.1	7.4
Valine	6.9	6.9	7.0	6.7	14.0
Methionine	1.3	2.0	1.5	1.6	1.7
Isoleucine	4.3	4.1	4.4	4.0	3.9
Leucine	8.4	7.6	8.4	7.6	7.6
Tyrosine	2.6	2.3	2.4	2.1	2.2
Phenylalanine	3.5	2.8	3.5	2.7	3.5

* Cystine and tryptophan not determined

TABLE 16. Amino Acid Composition of Gliadins (mole %)*

Amino Acid	Talbot	Manitou	Pembina	Stewart 63	Prolific Rye
Lysine	0.7	0.6	0.5	0.5	0.7
Histidine	1.4	1.4	1.6	1.6	1.3
Ammonia	39.8	41.0	39.1	40.3	37.3
Arginine	1.6	1.3	1.5	1.6	1.5
Aspartic acid	2.6	2.5	2.5	2.7	2.1
Threonine	2.2	2.0	2.1	1.9	2.0
Serine	5.3	4.7	5.5	4.7	4.9
Glutamic acid	38.8	40.0	40.0	39.3	36.7
Proline	17.5	18.5	17.5	17.6	20.3
Glycine	3.3	2.6	2.9	2.6	2.3
Alanine	3.0	2.7	2.9	3.0	2.5
Valine	4.2	4.0	4.0	4.1	9.6
Methionine	1.2	0.9	1.1	1.2	1.1
Isoleucine	3.8	3.9	3.8	4.1	3.2
Leucine	7.4	7.0	7.2	7.3	5.9
Tyrosine	2.1	1.7	1.9	2.1	1.0
Phenylalanine	4.8	5.3	4.9	4.9	4.6

*Cystine and tryptophan not determined

TABLE 17. Amino Acid Composition of Glutenins (mole %)*

Amino Acid	Talbot	Manitou	Pembina	Stewart 63	Prolific Rye
Lysine	1.0	1.0	1.1	1.1	2.0
Histidine	1.6	1.5	1.4	1.6	1.3
Ammonia	36.6	38.8	37.4	41.5	31.5
Arginine	2.1	1.9	2.0	2.3	1.7
Aspartic acid	2.5	2.3	2.2	2.5	2.7
Threonine	2.6	2.4	2.4	2.6	2.5
Serine	5.7	5.4	5.2	6.0	5.3
Glutamic acid	37.0	41.2	40.2	38.7	33.7
Proline	15.9	15.8	16.7	14.7	16.6
Glycine	6.3	6.0	5.6	6.0	7.2
Alanine	3.1	2.6	2.7	3.0	3.4
Valine	3.9	3.3	3.3	3.5	9.4
Methionine	1.2	0.6	0.9	1.2	1.0
Isoleucine	3.3	3.2	3.3	3.4	2.3
Leucine	7.0	6.1	5.8	6.3	5.2
Tyrosine	2.6	2.3	2.1	2.5	2.3
Phenylalanine	4.1	4.5	4.4	4.2	3.4

*Cystine and tryptophan not determined

TABLE 18. Amino Acid Composition of Residue Proteins (mole %)*

Amino Acid	Talbot	Manitou	Pembina	Stewart 63	Prolific Rye
Lysine	2.6	2.4	2.0	2.9	3.6
Histidine	1.7	1.7	1.6	1.7	1.8
Ammonia	30.5	30.2	32.5	29.5	26.9
Arginine	2.3	2.4	2.4	2.6	2.9
Aspartic acid	4.1	4.5	3.6	5.3	5.6
Threonine	3.3	3.3	3.2	3.6	3.7
Serine	5.9	6.2	6.5	6.6	5.5
Glutamic acid	32.0	31.8	34.5	29.7	26.4
Proline	12.2	11.9	12.5	10.9	12.4
Glycine	8.7	9.0	8.6	9.0	8.9
Alanine	4.8	4.8	4.2	5 5	6.2
Valine	5.0	4.8	4.3	5 0	5.6
Methionine	1.5	1.3	1.4	1.5	1.5
Isoleucine	3.4	3.3	3.2	3.6	3.2
Leucine	7 0	7.4	7.0	7 6	6.7
Tyrosine	1 7	1.9	1.8	1.5	1.6
Phenylalanine	3.5	3.4	3.3	3.4	3.8

*Cystine and tryptophan not determined

albumin and residue protein fractions and over 50 mole % in the gliadin and glutenin fractions. These fractions also had very low contents of the basic amino acids, and of methionine and tyrosine.

Discussion

The present results indicate that there is little variation in the amino acid composition of corresponding solubility classes in different classes of wheat and rye. The protein solubility fractions, with the exception of the globulins, contained high levels of glutamine and proline. Coulson and Sim (154) have previously shown that these proteins are rapidly degraded following germination and that a large parallel decrease of endosperm nitrogen takes place. Thus in wheat, and probably in other cereal endosperm proteins, the main function of the prolamines and glutelins (and probably the albumins), which together represent over 90% of the total endosperm protein, is to provide nutrition to the plant following germination. The high content of glutamine and proline in cereal endosperm proteins (155) would seem to indicate that the high incorporation of these amino acids has been favored by natural selection. This in turn suggests that these amino acids are most effective in providing nutritional elements to the growing seedling in the early stages of germination.

One obvious reason for the natural selection of high levels of glutamine in cereal storage proteins is its extra nitrogen atom in the amide form. Since amino acid amides are the main transport form of

organic nitrogen in plants (156), glutamine, the predominant transport form in most cereals, would provide an easily utilizable source of nitrogen to the growing seedling following germination. Incorporation of glutamine into endosperm proteins would segregate this transportable nitrogen into a temporary storage pool not available to metabolic systems of the embryo in the same way that endosperm starch serves as a storage pool of glucose. Utilization of these storage pools will not occur until protein and starch degrading enzyme levels are increased during germination.

Proline is also a principal amino acid of endosperm storage proteins. Pathways for the degradation of both proline and glutamine pass through glutamic acid which is deaminated either by transamination or oxidation to α-ketoglutarate, an intermediate of the Krebs cycle. The cyclic nature of the Krebs cycle requires that intermediates be present in sufficient amounts to maintain high respiration levels. Thus high levels of glutamine and proline in endosperm proteins may serve to prime and support high respiration levels in the seedling by providing a source of α-ketoglutarate. This hypothesis is consistent with the data of Lowe and Ries (152) who showed a strong positive correlation between endosperm protein content and the rate of seedling respiration following germination.

Another possible reason for natural selection of high proline and glutamine levels in cereal endosperm protein are their effects on solubility. The major endosperm protein fractions (prolamines and glutelins) have low solubilities. This effect is primarily due to the strong hydro-

gen-bonding of glutamine residues and the disruption of secondary structure by proline which favors aggregation of these proteins (30,157). Thus upon germination these proteins are not lost to the growing seedling through "leaching out" by water.

The proteins containing high proportions of glutamine and proline also have a low water binding capacity, primarily due to these amino acids. Bull (158) has shown that zein (corn prolamine) has a much lower water binding capacity than serum albumin or collagen. Low water contents in the dormant seed would preclude activation of enzymes necessary for germination. Low water contents would also lower the susceptibility of the proteins to bacterial and fungal attack and thereby favor higher stability during storage. Glutamine and proline may also be involved in other metabolic functions. Recently Gälsky and Lippincott (159) demonstrated that amino acids related to glutamic acid are able to induce α-amylase formation in barley aleurone suggesting additional regulatory functions of glutamine and proline in the germination process.

Thus more efficient storage of nitrogen and carbon skeletons to prime the Krebs cycle in an inert pool, insolubility and low water-binding capacity, and regulatory activity would seem to provide for natural selection of high levels of glutamine and proline in cereal endosperm proteins. Coincidently, this natural selection has produced a protein that has highly desirable functional properties in breadmaking.

ADDITIONAL DISCUSSION

The similarity in the calculated molecular weights of gliadin and glutenin subunits present in rye with those in wheat and its diploid progenitors supports cytogenetic evidence (108) that rye and the diploid progenitors of wheat were derived from a common ancestoral diploid. However significant physical and chemical differences in the properties of wheat and rye gliadins were found in the present study. Although gliadins from both wheat and rye could be separated by gel filtration into four distinct fractions with approximate molecular weights of greater than 100,000, and of 44,000, 27,000 and 10,000, the bulk of the rye gliadins had molecular weights greater than 100,000 while the majority of wheat gliadins were found in the third fraction (MW 27,000). Differences in the amino acid composition and SDS - PAGE patterns of corresponding fractions in wheat and rye indicated that the gliadins from these species have evolved in different directions. In rye the contents of glutamic acid and proline increased and that of the basic amino acids of aspartic acid and of glycine decreased as molecular weight increased. In wheat the three higher molecular weight fractions had similar amino acid compositions.

SDS - PAGE patterns of the gliadin fractions in wheat and rye showed that the three lower molecular weight fractions in both species consisted of single polypeptide chains, probably stabilized by intra-chain disulfide bonding. However the high molecular weight gliadin fraction in wheat consisted of smaller chains which were joined by inter-chain disulfide bonds to give proteins of higher molecular weight. In rye only a subunit(s) of MW - 108,000

was found.

Although qualitative differences in the SDS - PAGE patterns and amino acid compositions of corresponding gliadin fractions in the four wheat varieties studied were small, significant varietal differences in the distribution of the four gliadin fractions isolated by gel filtration were found. Substitution of these fractions for gliadin affected the mixing curves obtained with a synthetic dough system composed of glutenin, starch and gliadin. The high molecular weight gliadin fraction gave the stronger mixing curve while the lower molecular weight fractions gave weaker mixing curves. These results indicate that the molecular weight distribution of gliadin components may be important in determining mixing properties and possibly loaf-volume potentials of bread wheat flours. However further studies are needed to determine if the distribution of gliadin fractions varies in good and poor quality bread wheat flours. Previous studies by Mullen and Smith indicate that the molecular weight distribution of gliadins in short and long-mixing flours do vary (8).

Although the glutenin and residue proteins of wheat differ in their solubility properties, SDS - PAGE and electrophoretic patterns of these proteins isolated from a hard red spring wheat (cv. Manitou), following reduction and alkylation of disulfide bonds, were similar. These results indicate that the residue proteins are probably very high molecular weight glutenins which are characterized by more extensive inter-chain disulfide bonding. This conclusion is supported by previous studies (66-68). Several subunits present in gliadin also had molecular weights identical to those in glutenin and the residue proteins. Further studies are needed to determine if these subunits represent identical polypeptide chains.

CONTRIBUTIONS TO KNOWLEDGE

1) Wheat and rye gliadins, isolated by Osborne fractionation, were separated into four fractions of average molecular weights of about 100,000, 44,000, 27,000 and 10,000 by Sephadex-G100 chromatography in a strongly dissociating solvent (AUC). Significant intervarietal variation in the distribution of wheat gliadin fractions was found. In rye approximately 50% of the gliadin had molecular weights greater than 100,000.

2) The amino acid composition of the rye gliadin fractions showed a trend of increasing levels of glutamic acid and proline as molecular weight increased. The first three wheat gliadin fractions (MW's 100,000, 44,000 and 27,000) had amino acid compositions similar to whole gliadin while the fourth fraction (MW 10,000) had an amino acid composition similar to wheat albumin. Intervarietal variation in corresponding gliadin fractions was small

3) SDS-gel electrophoresis showed that rye gliadins have three subunits of MW's 110,000, 42,000 and 10,000. Three hexaploid wheat varieties had identical gliadin subunits with major subunits of MW's 10,000, 36,000, 40,000 and 50,000 and minor subunits of MW's 53,000, 78,000, 82,000, 108,000, 120,000 and 180,000. A tetraploid wheat variety lacked minor subunits of 88,000, 120,000 and 130,000 which are probably controlled by genes in the D genome.

4) Comparison of reduced and non-reduced wheat gliadin fractions by SDS-gel electrophoresis indicated that the high molecular weight fraction

is produced by inter-chain disulfide bonding of subunits with MW's 40,000, 50,000 and 53,000. The lower molecular weight gliadin fractions appear to be single chain polypeptide chains stabilized by intra-chain disulfide bonding.

5) Comparison of the gliadin and glutenin subunit structures of T. aestivum (AABBDD), T. monococcum (AA) and Ae. squarrosa (DD) showed that seven gliadin and nine glutenin subunits had identical molecular weights in all three species. Five gliadin and three glutenin subunits in T. aestivum were present in only one of its diploid progenitors. On the basis of this evidence, it appears that the majority of subunits present in T. aestivum are coded for by genes in more than one genome.

6) Three of four gliadin and eight of nine glutenin subunits present in S. cereale have molecular weights identical to subunits present in T. monococcum and Ae. squarrosa. These results indicate that S. cereale, T. monococcum and Ae. squarrosa are related phylogenetically.

7) Mixing studies of a synthetic dough system containing gliadin, glutenin and starch showed that the high molecular weight gliadin fraction of a hard red spring wheat variety (cv. Manitou) was more effective in strengthening the dough than two lower molecular weight gliadin fractions (MW= 44,000 and 27,000). These results indicate that the molecular weight distribution of the gliadin proteins in flour may be important in determining rheological properties.

8) Approximately 80% of the insoluble glutenin proteins (Osborne residue fraction) could be solubilized in 0.1N acetic acid after reduction and alkylation of disulfide bonds. The majority of these proteins were soluble in 70% ethanol (47%) and 0.1N acetic acid (35%) when sequentially

extracted with 0.5N sodium chloride, 70% ethanol and 0.1N acetic acid. Amino acid composition, electrophoresis and SDS-gel electrophoresis of the alcohol and acid soluble proteins showed that they resembled soluble glutenin.

9) The comparison of the amino acid composition of the Osborne solubility classes of three hexaploid wheat varieties and one tetraploid variety showed that corresponding solubility fractions had very similar amino acid compositions. A hypothesis for the natural selection of high levels of glutamic acid and proline in these proteins has been put forward.

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APPENDIX

Fig.13. Calibration curve for molecular weight determination for SDS-electrophoresis.

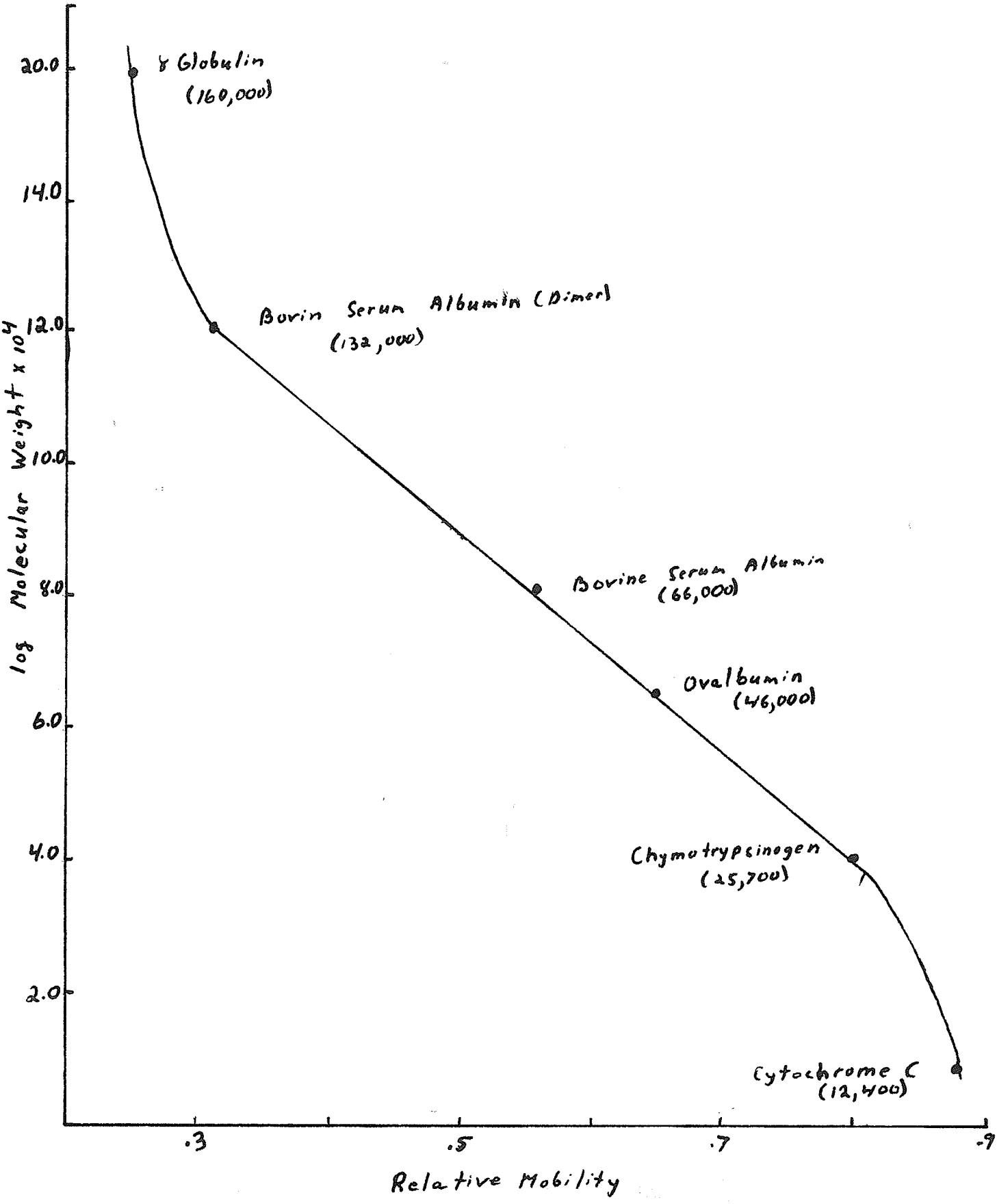
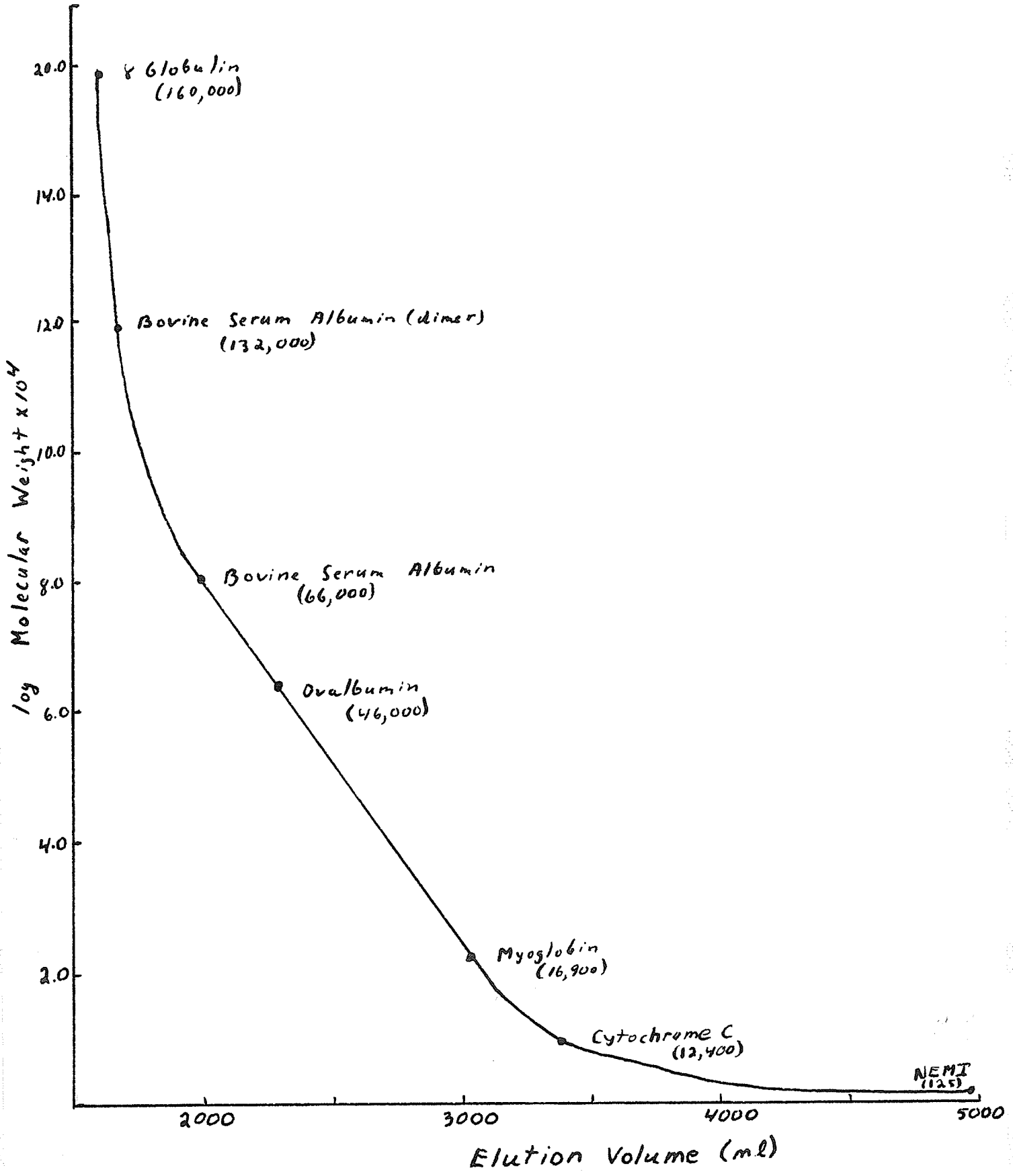

20.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
log Molecular Weight x 10^4
γ Globulin
(160,000)
Bovin Serum Albumin (Dimer)
(132,000)
Bovine Serum Albumin
(66,000)
Ovalbumin
(46,000)
Chymotrypsinogen
(25,700)
Cytochrome C
(12,400)
.3
.5
.7
.9
Relative Mobility

Fig.14 Calibration curve for molecular weight determination for Sephadex - G 100 chromotography in AUC.


γ Globulin
(160,000)
Bovine Serum Albumin (dimer)
(132,000)
Bovine Serum Albumin
(66,000)
Ovalbumin
(46,000)
Myoglobin
(16,900)
Cytochrome C
(12,400)
NEMI
(125)
20.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
log Molecular Weight x 10⁴
2000
3000
4000
5000
Elution Volume (ml)